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BY

R. W. MARSH

AND

I. THOMAS

WITH THE ASSISTANCE OF

F. J. ANSCOMBE J. T. MARTIN
F. C. BAWDEN D. J. WATSON

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CONTENTS OF VOL. 39, NO. 1

AN IMPROVED LABORATORY APPARATUS FOR APPLYING DIRECT SPRAYS AND SURFACE FILMS, WITH DATA ON THE ELECTROSTATIC CHARGE ON ATOMIZED SPRAY FLUIDS

BY C. POTTER

University of Rhode Island, Kingston, R.I., U.S.A., and the Department of Insecticides and Fungicides, Rothamsted Experimental Station, Harpenden, Herts

(With Plate 1 and 5 Text-figures)

A description is given of the design and physical performance of a laboratory spraying apparatus, which is an improved version of that described by the author in 1941. This apparatus is shown to be capable of giving good replication and an even distribution over a circular area 9 cm. in diameter with distilled water, a light petroleum oil and a heavy petroleum oil. It may therefore be used with a variety of media either for direct application to the organism, or for the application of residual films. With distilled water a difference in the environmental temperature of 20° F., between 60 and 80° F., produced approximately 10 % difference in the deposit and a difference of 20 % in the relative humidity of the environment between 60 and 80 % produced approximately 5 % difference in the deposit, so that it does not appear that closely controlled conditions, although desirable, are necessary for good replication.

A short investigation was made of the electrostatic effects, to try to determine whether variation in electrostatic charge could cause variation in weight of deposit. Tests using a copper spray target and a Perspex target indicated that electrostatic charge on the target itself was not important. It was found that the charge on the droplets of aqueous sprays varied considerably with the solute. In the presence of two non-ionic surface active agents the charge was greatly increased over distilled water, while in the presence of an anionic material it was reduced so that it could not be measured with the available apparatus. Other materials produced intermediate effects. The difference in charge did not appear to be due to differences in degree of atomization. In a given set of conditions a droplet of distilled water of average weight 0·00013 mg. at a potential of 25 V. carries a charge of 0·00026 e.s.u. and a droplet of 5 % v/v Lissapol N of average weight 0·000084 mg. at a potential of 49 V. carries a charge of 0·00044 e.s.u.

However, there appeared to be no correlation between the charge on the droplet, and the amount deposited, and this, together with further evidence obtained by applying potentials up to 1·5 kV. on the spray target, indicated that variations in electrostatic charge on the droplets were not likely to cause variation in the amount deposited, under the conditions of application in the apparatus.

It was concluded that the main cause of the variations of deposit that occurred in a series of measurements was likely to be difference in the amount of turbulence occurring in the spray tower, but that there were probably other sources that had not yet been recognized. The weight of deposit must therefore be checked constantly.

References are given to work illustrating the biological results obtainable with the apparatus.

INTRODUCTION

Various phases of work on the laboratory study of insecticides and fungicides call for an apparatus which will distribute a known amount of spray fluid evenly over a given surface area. Such types of apparatus are required for the study of the effects of direct application of contact insecticides, of the residual effects of contact poisons and for the investigation of the action of stomach poisons.

The purpose of this paper is to give an account of the developments in the design of an apparatus first described in 1941 (Potter, 1941), and to detail some experiments on the factors affecting the performance of this machine particularly on the electrostatic charges produced during atomization and their possible influence on the amount of deposition.

While the apparatus may be used for applying insecticides to foliage in order to study their stomach poison effect, and may also be used for the study of fungicides, it was primarily designed for the study of the action of contact insecticides, and in the body of the paper it will be considered in this connexion only.

The work was carried out at the University of Rhode Island, U.S.A.*, and the Rothamsted Experimental Station. An apparatus was constructed at Rhode Island State College in which a considerable number of modifications from the original design were incorporated and tested. With the experience gained from these tests, the final model was constructed at Rothamsted.

A description of both models is necessary to facilitate the description of the factors that were found to be important in affecting performance and for convenience in adjusting and working the apparatus.

There have been described in the literature a number of types of apparatus and technique for the laboratory study of the action of chemicals as contact insecticides. Summaries of information on the subject are given by Tattersfield (1939) and Campbell & Moulton (1943).

Some of these techniques are designed for the study of the action of chemicals when applied in a particular medium and by a particular process, for example, for the purpose of examining the effect of chemicals on a particular species or group of species of insects in a particular habitat, such as mosquito larvicides.

These methods are at present essential for particular fields of work, but are limited in their application. The more generalized methods consist of dipping the insects under test, treating individual test insects, spraying groups of test insects, and, since the importance of the residual effects of contact insecticides was first recognized (Potter, 1938), exposing insects on surfaces coated with films of insecticides.

Without going into details of the relative merits of these procedures, it may be said that one major disadvantage of dipping is that it limits the media that may be used, and that one major disadvantage of the methods available for treating

* During the course of a year's stay as visiting research professor of entomology.

individual insects is that it is difficult to treat a sufficient number of individuals to give quantitative data on the variations of response to a number of different chemicals.

The apparatus described in this paper is designed to give an even deposit of spray over a circular area about 9 cm. in diameter, and is thus suitable for studying the effect of chemicals both when applied as a direct spray and as a residual film. This apparatus is an improved version of that described in 1941 (Potter, 1941).

It may be used with a wide variety of media. Since the original description of its use with aqueous media, techniques have been described for its use with petroleum oils, either unmodified (Parkin & Green, 1943; Tattersfield & Potter, 1943), or in a modified form (Hewlett, 1947) with a specially designed atomizing nozzle (Hewlett, 1946).

It may thus be claimed that the apparatus is generally useful for the study of the action of insecticides.

Since the publication of the summaries referred to above, a number of methods have been published for the study of chemicals when applied as direct sprays and as residual films.

Apparatus for spraying insects has been described by Morrison (1943), Petty (1946), Monro, Beaulieu & Delisle (1947), Franzen (1948) and ten Houten & Kraak (1949). Of these, Morrison's apparatus is a slight modification of the improved Tattersfield apparatus, and the paper is mainly concerned with a very enlightening study of technique. The apparatus of ten Houten & Kraak could also be described as a modification of the Tattersfield apparatus, but the design of the atomizing nozzle is radically altered, to give, it is claimed, increased precision of adjustment and improved distribution. Data on the physical performance of the apparatus are given to substantiate these claims. It appears that neither the apparatus of Morrison nor that of ten Houten & Kraak will give an even distribution over a circle greater than 2·5 cm. in diameter.

The apparatus of Petty is designed for direct application and for deposition of residual films, but performance figures are given for a kerosene medium only and these do not include any data on evenness of distribution. The apparatus of Monro *et al.* is designed to apply residual films of insecticides in petroleum oils by directly spraying a surface, and data are given on the replication and distribution obtained. This apparatus would not appear to be suitable for use with aqueous media. No data are given of the physical performance of the apparatus described by Franzen, and in the absence of any details of the technique of treatment of the insects it is difficult to assess its comparative value as a laboratory instrument.

A number of types of apparatus for applying known deposits of insecticide evenly over a surface by a settling-out technique have been described. Apart from those dealt with in the summaries of Tattersfield and Campbell & Moulton, two recent designs are by Webb (1947) and Way (1949), both of whom give details of physical performance and a considerable amount of information on the factors affecting the

technique. An alternative technique for applying residual films has also been described recently by Parr & Busvine (1948). It seems, however, that these various types of apparatus are limited to the preparation of residual films, and even for this purpose may be restricted in their range of media and chemicals.

It is thought, therefore, that there is still the need for a general purpose instrument such as that described in this paper. It has been in continuous use, with various modifications, for more than ten years in the insecticides department at Rothamsted, and has been installed in a number of laboratories in this country and abroad.

General features of the apparatus

No modification of principle from the original design published in 1941 has been introduced, but the differences in constructional design are very large.

Pl. 1, fig. 1, shows a photograph of the intermediate design and Pl. 1, fig. 2, a photograph of the final design.

The apparatus consists of a reservoir and a specially designed atomizing nozzle which sprays down the centre of a metal tube on to a circular spray table. The atomizing nozzle is mounted so that it can be centred and the direction of the spray adjusted. The spray table has arrangements for levelling, centring and adjusting the gap between the table and the bottom of the tube or spray tower. The whole apparatus is mounted on a wooden stand with levelling screws attached to its legs.

The following is a description of the separate parts of the apparatus.

Atomizing nozzle

The form finally adopted (Pl. 1, fig. 4, and Text-fig. 1) is the same in principle as that first described, but has been modified to provide a mechanical adjustment for retracting and projecting the liquid jet, and an easier method for centring the liquid jet in the air jet.

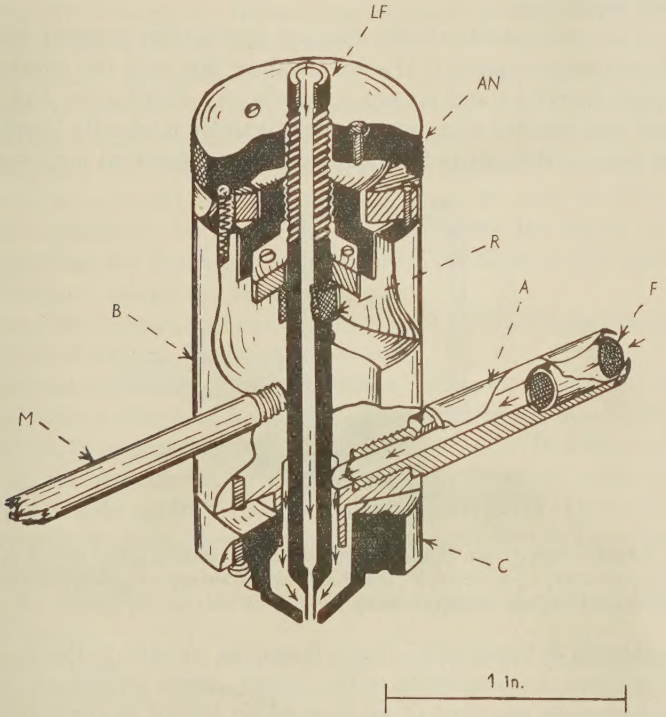
The dimensions of the liquid jet have been increased from 0·0135 to 0·030 in., to decrease the likelihood of blockage. The annular air space has been reduced, since it was thought that the reduced air flow would decrease the evaporation from droplets of volatile materials during their passage down the tower, and because it appeared from the experimental results that it resulted in improved replication of the deposit.

It had proved to be difficult to provide a satisfactory finish to liquid jets made of brass owing to the softness of the metal, and Monel metal was adopted for the later designs, since it is easily machinable but at the same time hard enough to provide a good finish, and is non-corrodable.

Hewlett (1946) published a description of an atomizing nozzle for this apparatus which was designed primarily for use with petroleum oils, with which medium it gives very satisfactory results. This nozzle has provision for the mechanical centring of the liquid jet in the air orifice, and for projecting and retracting the liquid jet.

The Hewlett atomizing nozzle has, however, a considerably larger annular air space, approximately 3·5 times more than the final design described here (0·0018 sq.in. as compared with 0·0005 sq.in.); it has a brass liquid jet and is considerably more bulky, being 2 in. in diameter instead of 1 in.

For the reasons described above, the design here described is preferred, particularly where the apparatus is likely to be used with a wide range of liquid media.



Text-fig. 1. Final design of atomizing nozzle. *A*, air lead; *F*, air filter; *C*, air cap; *LF*, liquid feed; *AN*, adjusting nut; *R*, rubber packing gland; *M*, mounting rod; *B*, body of nozzle.

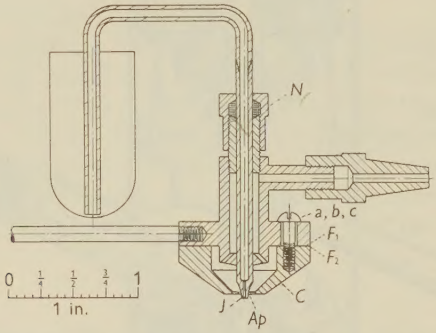
ten Houten & Kraak (1949) developed a nozzle, taking into account the previous designs of Potter (1941) and Hewlett (1946). This nozzle was designed to work with a different apparatus from that described here.

The ten Houten & Kraak nozzle has a number of interchangeable Monel liquid jets of different diameters to give good atomization of liquids of widely different physical characteristics. The vertical adjustment of the tip of the nozzle in relation to the air cap can be made very accurately by means of a calibrated adjusting nut, but no adjustment is provided for centring the liquid jet in the air jet. This nozzle

appears to embody several desirable features, but, as the authors state, it has to be made with the greatest accuracy owing to the lack of a centring adjustment.

The use of interchangeable liquid jets is not desirable with the apparatus described here, since any change in the diameter of the liquid jet produces a change in the distribution of the deposit. Furthermore, with liquid jets above a certain diameter, quite apart from difficulties of atomization, it does not appear to be possible to obtain an even distribution of the deposit and at the same time to have a satisfactory replication.

The use of interchangeable nozzles does not appear to be essential, since the data on physical performance given in Tables 3–8 show that with the nozzle described, having a single liquid jet and air cap, a satisfactory distribution and replication of the deposit was obtained using water, a light petroleum oil and a heavy petroleum oil and this range is thought to be adequate for most practical purposes.



Text-fig. 2. Intermediate design of atomizing nozzle. *N*, packing gland nut; *a*, *b*, *c*, adjusting screws for air cap; *C*, air cap; F_1 faced surface of air cap; F_2, faced surface of nozzle body; *J*, liquid jet; *Ap*, aperture of air cap.

The ten Houten & Kraak nozzle has a tangential air inlet to the air cap, which is stated to improve the distribution of the deposit. Some preliminary experiments were made here with this type of air flow which tended to confirm ten Houten & Kraak's results. However, there were indications that when used in this apparatus, errors of replication were considerably increased; the original co-axial air flow was therefore retained.

The first radical modification in design from the original nozzle is shown in Pl. 1, fig. 3, and Text-fig. 2. It is very similar to the Hewlett nozzle, except that it is very much smaller, the adjustments are not mechanical and the air flow is considerably less.

It is described here because it is very simple to make and will give a satisfactory performance. Also because it has been used for some of the investigational work.

The dimensions and general construction are shown in Text-fig. 2. Loosening the packing gland nut (*N*) allows the projection and retraction of the liquid jet.

Centring the liquid jet in the air jet may be accomplished by loosening the three screws *a*, *b* and *c*, and moving the air cap *C* in the appropriate direction. Movement is made possible by having a faced surface F_1 on the air cap sliding over a similar surface F_2 on the nozzle body, and by having oversize holes in the air cap for the screws *a*, *b* and *c*. The whole nozzle was made of brass, apart from the copper liquid feed tube, but there is no reason why the liquid jet should not be made of Monel metal which is to be preferred for this purpose. The internal diameter of the liquid jet (*J*) is 0·0275 in., the external diameter is 0·0374 in. The diameter of the aperture (*Ap*) in the cap is 0·0635 in.

The nozzle finally adopted is shown in Pl. 1, fig. 4, and Text-fig. 1. The liquid feed tube and the reservoir are omitted from Text-fig. 1. The liquid feed tube is made of stainless steel 0·0937 in. external diameter, and 0·073 in. internal diameter. It is 5 in. long and the bottom of the tube is 1 in. above the tip of the liquid jet. In Text-fig. 1 the scale and the relationships between the parts are shown. It has a mechanical adjustment for projecting and retracting the liquid jet, but the method of centring the liquid jet in the air jet is the same as that just described for the intermediate design and is manual.

Brass is used throughout, except for the liquid jet and feed tube which are made from Monel metal and stainless steel respectively.

Several methods of retraction and projection of the nozzle were tried out. The one finally adopted is shown in Text-fig. 1; although this was not incorporated in the nozzle at the time of the tests of physical performance, it differs only in convenience from that actually used. The mechanism consists of retraction or projection of the tip of the jet by turning the adjusting nut (*AN*). The rubber packing gland (*R*) prevents the body of the liquid jet from turning and allows sufficient up and down movement.

Centring the liquid jet in the air jet is carried out as described above for the intermediate design.

Although no detailed critical examination of the factors involved has been carried out, from the data already obtained it appears that the construction of the tip of the liquid jet, and the nature of the air flow round it are important factors in determining the evenness of atomization, and the distribution and replication of the deposits.

In the final design the liquid jet is shaped to a knife-edge at the tip and is polished externally to appear smooth under a low-power stereoscopic binocular microscope. The air cap venturi length is 0·050 in. The interior of the cap and the venturi is polished. The air flow is co-axial with the liquid jet.

This nozzle is somewhat more difficult to make than the intermediate design, but is easier to adjust. Owing to the small annular air space in this design it is essential to take precautions against any blockage in the air flow. A filter (Text-fig. 1, *F*) is inserted in the air intake of the nozzle. The filter is made from brass gauge of very fine mesh. An air flowmeter is also inserted in the airline to check any irregularities of air flow.

The critical measurements for the two nozzles are as follows:

	Intermediate design (in.)	Final design (in.)
Internal diameter of liquid jet	0·0275	0·030
External diameter of liquid jet	0·0394	0·050
Air cap orifice (diameter)	0·0635	0·056
Length of venturi	Knife edge	0·050

To set the nozzle the liquid jet is adjusted until the tip of the jet is flush with the outer surface of the air cap. The air cap is then adjusted so that the liquid jet is centrally placed in the air jet. These adjustments should be made under a stereoscopic binocular microscope.

Small movements of projection or retraction of the liquid jet alter the rate of delivery and degree of atomization of liquid and hence the deposition and distribution. The best setting must be obtained experimentally.

Atomizing nozzle mounting (Pl. 1, figs. 3, 4)

The mounting originally described was later considered undesirable, since the framework consisted of rectangular brass strip $\frac{3}{8}$ in. wide which might interfere with the smooth air flow down the tower. An intermediate design is shown in Pl. 1, fig. 3. The design was satisfactory in that the framework consisted of a small diameter brass rod which permitted smooth air flow, and it furthermore permitted large adjustments for experimental purposes. The design, however, was somewhat complicated and it was found that when the atomizing nozzle and spray tower were accurately constructed, large adjustments were not necessary, so the simplified design shown in Pl. 1, fig. 4, was finally adopted.

This mounting consists of three mild steel rods of $\frac{3}{16}$ in. diameter and $4\frac{1}{2}$ in. long, which are screwed $\frac{1}{4}$ in. into the body of the atomizing nozzle so that they project at angles of 120° to each other. Each bar is attached to the upper flange of the spray tower by brass terminals of the type containing eye-holes. The bars pass through the eyes of the terminals, and by adjusting the length of bar between the terminal and the atomizing nozzle body, the atomizing nozzle can be centred in the top of the spray tower. The base of the terminal is bolted to the flange of the spray tower, and may be raised or lowered, thus permitting adjustment of the direction of the atomized jet down the spray tower. Three radial slots ($\frac{3}{16} \times \frac{1}{2}$ in.) are made in the tower flange to allow lateral movement of the terminals when centring the atomizing nozzle.

This simple arrangement gives ample movement for adjusting the position and direction of the spray jet and at the same time is rigid.

The spray tower (Pl. 1, figs. 1, 2)

Only very minor alterations have been made to the original measurements of the spray tower, but some information has been gained on the necessity for its accurate construction.

The tower consists of a cone-shaped upper portion and a parallel-sided lower portion.

When a tube of this kind is made by the usual process of lap-jointing the metal sheet, some distortion of symmetry invariably occurs. The tube is flattened on the side of the joint, instead of being circular in cross-section, and the upper cone-shaped part of the tube is not symmetrically placed on the parallel-sided lower part.

In the intermediate design shown in Pl. 1, fig. 1, where the tube was lap-jointed, the difficulty of the asymmetrical position of the cone on the parallel-sided part was partially overcome by constructing the tube in two parts so that the cone could be adjusted with respect to the parallel-sided part of the tube.

A series of distribution studies showed that if the cone was not symmetrically placed on the lower part of the tube, it was very difficult indeed to get an even distribution.

For the final design, therefore, a tube was made by first preparing an accurate former. The sheet was then cut to size and rolled. Finally it was fitted round the former, clamped and butt-welded.

The flanges at the top and bottom of the tube are of 10-gauge mild steel, cut separately and silver soldered on to the 20-gauge mild steel tube previously formed. The completed tube is then chromium plated.

The measurements of the tube finally adopted are as follows:

(1) Overall length 27 in.

(2) Length of conical section $13\frac{1}{2}$ in. with diameter at top $6\frac{1}{4}$ in. tapering to $4\frac{3}{4}$ in.

(3) Length of parallel-sided section $13\frac{1}{2}$ in. of uniform $4\frac{3}{4}$ in. bore.

(4) The top and bottom flanges $1\frac{1}{4}$ in. wide.

In addition to the radius slots for the nozzle mounting, the top flange is fitted with three adjusting screws at equal intervals round its perimeter. The tips of these three screws rest in metal cups on top of the apparatus stand and are used for levelling the tube.

The spray table and universal adjustment (Pl. 1, figs. 1, 2, and Text-fig. 3)

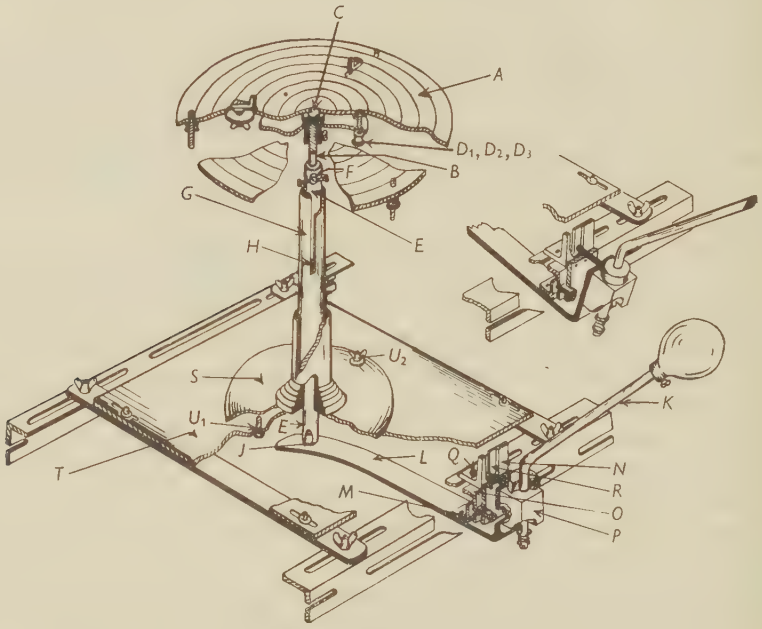
Underneath the bottom of the tower is a circular plate, the spray table.

The material to be sprayed is placed on the spray table for treatment. Since the gap between it and the bottom of the tower in the spraying position is not wide enough to permit easy placing and removal, the table has therefore to be moved up and down to facilitate this process. At the same time the position of the spray table and the gap between it and the bottom of the tower is critical in determining the amount and distribution of the spray deposit, so that an accurate and reliable mechanism for adjusting the position of the plate and enabling it to be lowered and raised again to exactly the same position is a necessity.

The intermediate design of mechanism for this purpose is shown in Pl. 1, fig. 1. This design proved efficient, but was rather clumsy. The final design is shown in

Pl. 1, fig. 2 and Text-fig. 3, and proved to be both neat and efficient. It involves very large changes from the original design.

The spray table, Text-fig. 3 (*A*), consists of a circular brass plate $6\frac{3}{4}$ in. diameter and $\frac{1}{8}$ in. thick with three screws equidistantly placed about its perimeter. It is centrally mounted on a silver steel rod $\frac{1}{4}$ in. in diameter and $4\frac{1}{2}$ in. long (*B*) through



Text-fig. 3. Spray table and universal adjustment. *A*, spray table; *B*, silver steel mounting and adjustable rod; *C*, universal joint; D_1, D_2, D_3, adjustment nuts of the universal joint; *E*, brass lifting pillar; *F*, grub screw on to the adjustment rod; *G*, sleeve for the lifting pillar; *H*, guide screw; *J*, ball-bearing surface; *K*, handle of lever; *L*, lever; *M*, hinge of lever; *N*, stop mechanism; *O*, slotted angle bar to which the lever is attached; *P*, bearing block for the handle of the lever; *Q*, mild steel rod of the stop mechanism; *R*, bearing surface of the stop mechanism; *S*, base of the spray table mounting; U_1, U_2, adjusting nuts for the base of the spray table mounting; *T*, main base plate.

a universal joint (*C*) consisting of a $\frac{1}{2}$ in. diameter steel ball-bearing clamped between the centre of the bottom of the table and a brass receptacle on the top of the $\frac{1}{4}$ in. rod (*B*). The table is levelled by manipulating the three knurled nuts (D_1, D_2, D_3) of the universal joint. By means of this adjustment the level of the table can be adjusted so that the gap between the table and the bottom of the spray tower is equal all round; the distance screws are then set so that the adjustment is maintained and any deviation may be noted.

The $\frac{1}{4}$ in. rod (*B*) is set in a hole of the same diameter drilled in a solid brass rod (*E*) $\frac{5}{8}$ in. in external diameter and 7 in. long. The distance that the $\frac{1}{4}$ in. rod (*B*) is inserted into the hole in the $\frac{5}{8}$ in. rod is adjusted by means of the screw (*F*) and this distance governs the gap between the top of the table and the bottom of the spray tower.

The rod (*E*) is placed in a sleeve consisting of a brass tube (*G*) $\frac{3}{4}$ in. in external diameter and $4\frac{1}{2}$ in. long in which it is on a sliding fit. The tube has a slot 2 in. long and $\frac{3}{32}$ in. wide which takes a guide screw (*H*) set in the rod (*E*).

In the bottom of the rod (*E*) is set a $\frac{1}{2}$ in. steel ball (*J*) which acts as the bearing surface of the rod on one end of the lever (*L*) by which the rod and hence the table is moved up and down.

The pivot of the lever consists of a hinge (*M*) attached to the lever (*L*) and to the slotted angle bar (*O*) by means of which the whole mechanism is attached to the frame of the apparatus.

The handle of the lever (*K*) is made to rotate in a horizontal plane by setting it in a bearing block (*P*) close to the point of pivot.

Moving the handle to the right allows a short mild steel rod (*Q*) to pass through a slot in the stop mechanism (*N*). The handle is then raised and the table is lowered.

The table is brought up into the spraying position and held there by depressing the handle, and then moving it to the left so as to bring it back to the central position when the steel rod (*Q*) is brought up against the bearing surface of the stop mechanism (*N*) at face (*R*).

The position of the entire lever mechanism can be adjusted by moving the pivotal portion along the slot in the angle bar (*O*), thus allowing the bearing surface of the lever always to be centrally placed on the steel ball at the base of the rod (*E*).

In order that the spray table can be centred beneath the spray tower the tube (*G*) is mounted on a base plate $4\frac{1}{2}$ in. in diameter made of 10-gauge brass (*S*) which can slide over the surface of the 8 in. sided square 10-gauge brass main base plate (*T*).

The details of construction are shown in Pl. 1, fig. 2 and Text-fig. 3.

In the centre of the main base plate is a circular hole $1\frac{1}{2}$ in. in diameter which permits the passage of the rod (*E*), and in the sliding base plate (*S*) are two diametrically opposed $\frac{1}{2}$ in. holes approximately $\frac{1}{2}$ in. in from the perimeter of the plate, through which project two 2 BA brass screws U_1, U_2 set in the main base plate. This arrangement permits universal movement in a horizontal plane of the sliding base plate (*S*) over the main base plate (*T*), and hence the centring of the spray table beneath the spray tower. When the correct adjustment has been made, the sliding base plate is firmly fixed in place on the main base plate by tightening the wing nuts on the two 2 BA screws (U_1, U_2) on to two washers covering the $\frac{1}{2}$ in. holes in the sliding base plate.

The main base plate (*T*) is bolted on to an angle iron frame, which is itself screwed on to the main stand holding the whole apparatus.

The stand

The stand (Pl. 1, fig. 2) is a rectangular wooden structure 41 in. high by 15 × 15 in. Both upper and lower cross-braces and the uprights are of 2 × 2 in. timber.

The tower is suspended by its top flange, adjusting screws operating in three metal cups fixed to the perimeter of a 10 in. hole cut in a five-ply board screwed to the top of the stand.

The main base plate frame is screwed to the four uprights just above the lower cross-braces and the complete stand fitted with four levelling screws.

Two additional cross-braces are screwed to the upright 15 in. above the lower cross-braces. These braces carry a flat brass ring, external diameter 10 in., affixed to which are three mounted brackets fitted with screws which may be adjusted to impinge upon the tower wall; this enables the tower to be held firmly once its position has been properly adjusted. A pair of auxiliary cross-braces are attached 10 in. below the upper cross-braces in order to strengthen the frame.

Setting up the apparatus for use

The stand is levelled by placing a spirit-level on the top platform and adjusting the levelling screws on the legs.

The central axis of the spray tower is then adjusted to the vertical by dropping a plumb line from the centre of the top of the tower and adjusting the levelling screws on the top flange of the tower so that the plumb-bob is over the centre of the bottom of the tower. The centres are found by means of cardboard circles of the same diameter as the top and bottom of the tower respectively.

By means of the adjustments already described in the section on the spray table and universal adjustment (pp. 9–11), the spray table is then centred beneath the spray tower and its height and level adjusted so that when in the spraying position it is the correct distance from the bottom of the spray tower with the gap even all the way round. In the model described a $\frac{1}{2}$ in. gap gives an even distribution of the liquids tested, at convenient spraying pressures.

The nozzle, which has been adjusted previously so that the liquid jet is centrally placed in the air jet, and the approximate position of the tip of the jet set in relation to the air cap as described in the section on the atomizing nozzle, is centred in the top of the tower by first slackening the three rod-clamping screws and the three lower terminal nuts, and then adjusted so that the length of all three arms of the atomizing nozzle mounting is equal. It is then adjusted simply by eye so that it is spraying vertically down the tower.

To obtain the best distribution, the final adjustments of the position of the nozzle and the tip of the liquid jet are made by trial and error. The weight of spray on cover-slips distributed over a plate is obtained and adjustments made until there is the minimum difference in weight of deposit when any one cover-slip is compared with another.

Air pressure

The air pressure must be kept as constant as possible. It has been found that satisfactory results can be obtained with working pressures of from 15 to 80 cm. Hg. The pressure should not vary more than 0·5 cm.

A fairly constant air pressure may be obtained by using an air compressor and large reservoir in which the air is compressed—at 50–60 lb./sq.in. (258·5–310·3 cm. Hg). The required pressure at the nozzle is then obtained by means of reducing valves preferably in at least two stages. The final adjustment may best be obtained by means of a needle valve.

A good air blower is a satisfactory source of air at constant pressure, but the maximum pressure is from 12 to 15 lb./sq.in. (62·05–77·56 cm. Hg).

The most satisfactory pressure-indicating device would appear to be an open mercury manometer. A differential pressure air flow-meter should be put in the air line just before the atomizing nozzle to check any irregularities in air flow.

Method of use

The required deposit per unit area is first obtained by spraying on to a dish or plate of known area and weighing, then adjusting until the correct deposit is obtained.

Major alterations in weight of deposit are obtained by altering the amount placed in the reservoir, the final adjustments being made by altering the air pressure. The deposit is inversely related to the air pressure.

In operation the spray fluid is measured out from a pipette into the reservoir of the atomizer. The spray table is lowered and the material to be sprayed is placed on it and the table then returned to the spraying position. The compressed air is then turned on and the fluid sprayed down the tower. When all the fluid has been sprayed, the compressed air is turned off, the table lowered, and the sprayed material removed.

Physical performance

Some data on the physical performance of the intermediate design are set out in Tables 1 and 2.

The actual spraying performance of this model is no better than the original design, almost certainly owing to imperfections in the shape of the spray tower and lack of finish in the liquid jet and air cap of the nozzle. This model, therefore, is an improvement only in so far that it is mechanically much easier to set up and operate, the nozzle adjustments in particular being simpler and more reliable.

Data on the physical performance of the final version of the apparatus using three test fluids, viz. water, a light petroleum oil and a heavy petroleum oil are given in Tables 3–8.

TABLE 1. *Distribution of deposit given by the intermediate design of apparatus*

(Measured by obtaining the weight of water deposited on cover-slips disposed about a ground glass plate of 9 cm. diameter. Gap, 0·25 in.; temperature, 77° F.; R.H., 74 %, 5 c.c. in reservoir; air pressure, 14·5–15·5 cm. Hg.)

Test no.	Position of cover-slip 1 Centre	2 Left rear	3 Right rear	4 front centre	Mean (mg.)
1	45·5	46·5	46·5	41·5	45·0
2	42·0	45·5	42·5	44·0	43·5
3	44·0	45·0	42·5	46·5	44·5
4	46·0	48·5	46·0	46·5	46·75
5	46·0	46·5	46·0	45·0	45·70
Means	44·7	46·4	44·7	44·7	45·09

S.E. of a single deposit = 1·52 ≡ 3·37 % of mean. S.E. of the mean of five deposits at one position = 0·6782. S.E. of the mean of four deposits at one trial = 0·7583. No significant difference in deposit over area of glass plate.

TABLE 2. *Replication of deposit on a 9 cm. Petri dish*

(Conditions as in Table 1.)

Test no.	Deposit (mg.)	Test no.	Deposit (mg.)	Test no.	Deposit (mg.)	Test no.	Deposit (mg.)	Test no.	Deposit (mg.)
1	614	3	633	5	664	7	649	9	626
2	613	4	614	6	647	8	624	10	617

Mean total deposit = 630·1 mg., S.E. = ± 5·61.

(*a*) *Distilled water*

The distribution was determined by weighing the deposits on circular cover-slips 2·56 cm. in diameter, one of which was placed in the centre and three equally spaced round the periphery of a 9 cm. glass plate.

The replication was estimated from a series of determinations of the deposition on a 9 cm. diameter Petri dish.

TABLE 3. *Distribution of distilled water given by the final design of apparatus*

(Conditions of test: room temperature, 70° F.; R.H., 56 %; air pressure of atomization, 69·5 cm. Hg; gap between the spray table and bottom of the tower, 0·5 in.; volume of fluid in the reservoir, 5 c.c.; time taken to spray, 8 sec.; deposit in mg. on glass cover-slips, 2·56 cm. diameter.)

Position 1 (centre)	Position 2 (periphery)	Position 3 (periphery)	Position 4 (periphery)	Mean
32·5	33·2	33·7	34·0	33·35
31·4	31·9	32·3	33·1	32·18
34·6	34·3	33·9	35·7	34·62
33·9	33·1	34·0	34·2	33·80
34·8	34·3	34·2	33·5	34·20
Mean 33·44	33·36	33·62	34·10	33·63

S.E. of a single deposit = 0·6148 = 1·83 % of the mean. S.E. of the mean of five deposits at one position = 0·275. S.E. of mean of four deposits at one trial = 0·307. These figures show that there is no significant departure from evenness of deposit over the area tested.

The figures for replication of deposit of distilled water set out in Table 4 show a considerable improvement on those obtained with previous designs, and this is

thought to be mainly due to the increased accuracy in the construction of the apparatus.

TABLE 4. *Replication of deposit of distilled water given by the final design of apparatus under the same conditions of test as in Table 3*

Test no. ...	1	2	3	4	5
mg./dish 9 cm. diam.	367	371·5	364	364	362
mg./sq.cm.	5·7668	5·8389	5·7210	5·7210	5·6896
Test no. ...	6	7	8	9	10
mg./dish 9 cm. diam.	362	364·5	357	358·5	365
mg./sq.cm.	5·6896	5·7288	5·6110	5·6435	5·7367

Mean total deposit = 363·5 mg., S.E. ± 4·106. Mean deposit per sq.cm. = 5·714 mg., proportional S.E. = ± 1·129 %.

(*b*) *Light petroleum oil* (*odourless distillate*)

The specification of the oil used is as follows: sp.gr. 0·782–0·785; distillation range: 12 % at 210° C., 38 % at 220° C., 65 % at 230° C., 85 % at 240° C., 95 % at 250° C.; flashpoint by open cap method, 187–203° F.; the oil is entirely unsulphonatable and consists therefore of pure paraffin hydrocarbons.

The figures for distribution and replication were obtained by the same technique as that used for distilled water and are given in Tables 5 and 6.

The figures in Table 5 show that there is no significant departure from evenness over the area tested.

TABLE 5. *Distribution of a light petroleum oil given by the final design of apparatus*

(Conditions of test: room temperature, 66–68° F.; R.H., 60–61 %; air pressure of atomization, 73 cm. Hg; gap between the spray table and the bottom of the tower, 0·5 in.; volume of fluid in the reservoir, 5·0 c.c.; time taken to spray, 10 sec.; deposit in mg. on glass cover-slips, 2·56 cm. diameter.)

Position 1 (centre)	Position 2 (periphery)	Position 3 (periphery)	Position 4 (periphery)	Mean
16·0	16·2	15·9	16·8	16·22
16·3	16·1	16·8	16·4	16·40
16·4	15·9	17·1	16·8	16·55
16·3	17·2	15·9	16·1	16·37
15·6	15·8	16·1	15·2	15·67
Mean 16·12	16·24	16·36	16·26	16·24

S.E. of a single deposit = ± 0·4854 or 2·99 %. S.E. of the mean of five deposits at one position = ± 0·2171. S.E. of the mean of four deposits at one trial = ± 0·2427.

TABLE 6. *Replication of the deposit of a light petroleum oil given by the final design of apparatus under the same conditions of test as in Table 5*

Test no. ...	1	2	3	4	5
mg./dish 9 cm. diam.	188·8	186·4	197·7	187·5	187·8
mg./sq.cm.	2·9676	2·9299	3·1075	2·9472	2·9519
Test no. ...	6	7	8	9	10
mg./dish 9 cm. diam.	195·6	198·2	191·4	187·0	193·6
mg./sq.cm.	3·0745	3·1154	3·0085	2·9393	3·0431

Mean total deposit = 191·40 mg., S.E. = ± 4·567. Mean deposit per sq.cm. = 3·0085 mg., proportional S.E. = ± 2·39 %.

(*c*) *Heavy petroleum oil* (*Wakefield half white oil*)

The specification of the oil used is as follows: 10 % distilled at 298–319° C., 80 % at 319–388° C.; flashpoint (closed) = 310° F.; viscosity, Redwood 1 at 70° F. = 104 (33·2 centistokes at 20° C.); sp.gr. at 15·5° C. = 0·880; unsulphonatable residue, 88 % by volume.

The figures for distribution and replication were obtained by the same technique as that used for distilled water and the light petroleum oil and are given in Tables 7 and 8.

The figures in Table 7 show that there is no significant departure from evenness of deposit over the area tested.

TABLE 7. *Distribution of a heavy petroleum oil given by the final design of the apparatus*

(Conditions of test: room temperature, 67·1–69·8° F.; R.H., 55–57 %; air pressure of atomization, 73 cm. Hg; gap between the spray table and the bottom of the tower, 0·5 in.; volume of fluid in the reservoir, 5·0 c.c.; time taken to spray, 55–57 sec.; deposit in mg. on glass cover-slips, 2·56 cm. diameter.)

Position 1 (centre)	Position 2 (periphery)	Position 3 (periphery)	Position 4 (periphery)	Mean
10·0	10·0	9·9	9·3	9·80
10·1	9·9	10·1	9·7	9·95
9·8	10·0	10·2	10·0	10·00
9·8	10·1	10·1	10·0	10·00
10·1	9·8	9·8	10·0	9·92
Means 9·98	9·98	10·02	9·80	9·93

S.E. of the means of a single deposit = ±0·2090 or 2·10 %. S.E. of the mean of five deposits at one position = ±0·0935. S.E. of the mean of four deposits at one trial = ±0·1045.

TABLE 8. *Replication of the deposit of a heavy petroleum oil given by the final design of the apparatus*

(Conditions of test were the same as in Table 7 with the exception that: room temperature, 68–68·4° F.; R.H., 64 %; time taken to spray, 60·5–64 sec.)

Test no. ...	1	2	3	4	5
mg./dish 9 cm. diam.	111·0	113·1	110·0	114·5	112·2
mg./sq.cm.	1·7447	1·7777	1·7295	1·7997	1·7636
Test no. ...	6	7	8	9	10
mg./dish 9 cm. diam.	112·9	111·5	112·6	113·0	111·9
mg./sq.cm.	1·7746	1·7526	1·7699	1·7762	1·7589

Mean deposit = 112·27 mg., S.E. of mean = ±1·256. Mean deposit per sq.cm. = 1·7647 mg., proportional S.E. = ±1·13 %.

The figures given in Table 8 show that a satisfactory replication of deposit can be obtained with a heavy petroleum oil.

Discussion on physical performance

Judging from the figures given in Tables 3–8, the performance of this model is somewhat better than the original design, and this is achieved using a liquid jet of larger diameter than that used in the original model. This is important, since it decreases the probability of a blockage in the nozzle, and blocking is one of the major weaknesses of any spraying apparatus using atomizing nozzles.

The figures given above were obtained without using any special precautions such as providing a constant environment, and may therefore be regarded as giving a fair sample of performance under average working conditions. The setting to obtain even distribution was quickly obtained. It must, however, be added, that at least with aqueous media, if spraying takes place over a period of 2 or 3 hr., the weight of the mean deposit may change, for reasons that have not been ascertained. This drift is normally not large, but it may bring the overall variation up from 6–8 % to 12–15 %. It may, of course, be prevented by checking the deposit at intervals throughout the spraying.

One disadvantage of the present design is the very small annular air space of the atomizing nozzle which was found to be necessary to obtain adequate distribution with the large aperture liquid jet. Any variations in air flow will cause considerable variation in the deposit, so that adequate precautions must be taken to keep the air line clean.

Where solutions or dilute fine suspensions only are to be used, the dimensions for air and liquid orifices given for the intermediate design of the nozzle may be preferable, since they give adequate distribution and replication, and less attention needs to be paid to the cleanliness of the air line.

When all these figures are taken into consideration it may be said that the apparatus will give satisfactory distribution and replication of the deposit with three test fluids typical of those most frequently used as media for insecticides. No alterations in the set up of the apparatus need be made when changing from one fluid medium to the next.

Factors influencing the variation of deposit

In spite of the considerable care taken in the construction and operation of this apparatus, some variation in the replication of the deposit was found to occur, more particularly with aqueous media, and an effort was made to examine some possible sources of this variation. Three possible sources were considered: (*a*) environment (temperature and humidity), (*b*) electrostatic charge, (*c*) turbulence.

(*a*) *Environment*

For the purpose of this study the apparatus was set up in a room where the temperature and humidity could be controlled, and some data were obtained on the

effect of variations of temperature and humidity on the deposit, using distilled water as the test fluid. The data are set out in Table 9.

TABLE 9. *Effect of variation of temperature and humidity on the deposit using distilled water as the test fluid*

(Air pressure of atomization, 69·5 cm. Hg; gap between the spray table and the bottom of the tower, 0·5 in.; volume of fluid in the reservoir, 5·0 c.c.; deposit, mg./cm. diameter Petri dish.)

	Test no.							
R.H. (%)	1	2	3	4	5	Mean	S.E.	% S.E.
				(*a*) Temperature, 80° F.				
80	387	368	381	360	366	372·4	±11·1937	3·01
60	364	350	346	353	344	351·4	± 7·8613	2·24
50	354	340	346	337	359	347·2	± 9·2574	2·67
				(*b*) Temperature, 60° F.				
80	398	404	408	400	412	404·4	± 5·7271	1·42
60	384	361	386	381	389	380·2	±11·1221	2·93

Table 9 shows that while variations in temperature and humidity affect the deposit of distilled water to some extent, the indications are that only large variations of the order of 20° F. or 20 % R.H. produce significant differences. It appears, therefore, that small differences in temperature and humidity will not affect the performance of the apparatus, and that temperature and humidity effects are not likely to be an important cause of the variations of replication that have been found. It follows from this also that the apparatus should give a satisfactory performance in the absence of any elaborate precautions for maintaining a constant environment.

(*b*) *Electrostatic effects*

In the publication first describing this apparatus (Potter, 1941), an account is given of experiments which showed that unless the spray tower consisted of an earthed conductor, a considerable charge could be built up on it, due to the deposition of atomized droplets carrying a charge. If the spray tower consists of an earthed metal tube then it would seem that this can no longer serve as a source of variation, and there remains the effect of any possible variation in the charge on the droplets reaching the spray target or in the charge on the spray target itself.

If this last possibility is considered first, it seems feasible that the glass Petri dish in which the deposit is collected may become charged to a varying degree when it is dried between treatments, and thus cause variations in the deposit on it.

For convenience a Perspex plate was used for the insulating target which would retain a charge, and this was compared with a copper plate of the same size. A series of measurements of the deposits on these two targets was made to determine if there was any difference in the weight or variation of the deposit when the insulating surface was compared with the earthed metal conductor.

The experiment consisted of measuring the deposits, first on a circular copper plate 9 cm. in diameter which was earthed, and secondly on a circle of Perspex of the same diameter which was placed on porcelain insulators. Eighteen measurements in all were taken in alternate sets of three, the conductor alternating with the non-conductor. The results are set out in Table 10. The average deposit on the copper plate exceeds that on the Perspex by $2{\cdot}30 \pm 16{\cdot}15$ mg. The difference is therefore not significant.

TABLE 10. *Comparison between the deposits on an insulating surface and those on a conducting surface at earth potential*

(Material, distilled water; air pressure, 72·0 cm. Hg; amount in reservoir, 5 c.c.; temperature, 16·6–17·5° C.; gap, 0·5 in.; R.H., 60–62 %; targets: (1) copper plate 9 cm. diameter kept at earth potential, (2) Perspex plate 9 cm. diameter insulated from conducting surfaces.)

Spray (sec.)	Spray no.	Deposit (mg.)	Spray (sec.)	Spray no.	Deposit (mg.)
8·5	1	646·2	8·5	4	642·5
8·5	2	648·2	8·5	5	656·7
8·5	3	661·0	8·5	6	642·9
8·25	7	632·9	8·5	10	641·0
8·5	8	651·6	8·5	11	627·6
8·5	9	626·0	8·5	12	632·9
8·5	13	644·3	8·5	16	643·5
8·75	14	613·5	8·25	17	615·0
8·25	15	637·4	8·5	18	639·2
	Mean deposit	640·12		Mean deposit	637·82

The evidence of this experiment is that when deposits are collected under conditions where a charge can build up on the receiving plate, and under conditions where the receiving plate is kept at earth potential, no significant difference in deposit occurs, i.e. the effect of any build up of charge is negligible. This also shows that any electrification during the drying of the Perspex plate had negligible effect. There remains the second possibility that variations in deposit are due to the variation in the charge present on the atomized droplets.

When distilled water is atomized the droplets are found to be charged. The very fine droplets are oppositely charged from the larger droplets (Nolan, 1914, and others). Following earlier work on this subject, Wampler & Hoskins (1939) have shown, and it was confirmed by Potter (1941), that dissolved or emulsified substances could produce large variations of charge on droplets according to the nature and amount of the substance present in the spray medium. It is possible, therefore, that the charge on the particles may be an important factor governing the amount of deposit.

It did not appear easily possible to decide from theoretical considerations the amount and variation of charge that would affect deposition, particularly when the droplets were being impacted by a stream of air and not by gravitational forces alone.

Some experiments were therefore made to determine the charge on the atomized droplets and to obtain evidence if variation of this charge was correlated with differences in deposition.

At first it was found to be difficult to obtain reproducible results, probably owing to leaks developing in the insulation, due to films of moisture. However, by using a series of glass-crystallizing dishes separated by sealing wax rods (Text-fig. 4), a satisfactory insulator was produced. The charge was measured on a calibrated gold leaf electroscope with scale and magnifying lens. Text-fig. 4 is a diagram of the arrangement used for the measurements.

Measurements were first made with distilled water, but it was subsequently found that the presence of surface active agents commonly used in insecticidal sprays could alter the amount of charge considerably. Measurements of the sign and approximate comparative amount of charge were made of solutions of a number of materials and the results are set out below (Table 11). The sign of the charge was determined by putting a charge of known sign on the electroscope by means of a dry battery, spraying the solution on to the target which was connected to the electroscope, and noting what effect it had on the deflexion. Each solution was checked against both a positive and negative charge.

TABLE 11. *Sign and approximate relative amount of electrostatic charge on droplets of distilled water and solutions of surface-active agents*

(Conditions of test: spray pressure, 73 cm. Hg; target circular metal receiver, 6 cm. diameter with wall 1·6 cm. high; capacity of system, 10·6 μF.)

Test fluid	Sign of charge	Amount of charge*
1. Distilled water	Positive	+ +
2. 5 % v/v Lissapol N†	Positive	+ + + + +
3. 0·2 % v/v Lissapol N†	Positive	+ + + +
4. 5 % v/v Triton N 100‡	Positive	+ + + + +
5. 0·2 % v/v Triton N 100‡	Positive	+ + + +
6. 5·0 % w/v Saponin	Positive	+ + +
7. 0·2 % w/v Saponin	Positive	+
8. 5·0 % v/v triethanolamine lauryl sulphate	Positive	+ + +
9. 0·2 % v/v triethanolamine lauryl sulphate	Positive	+
10. 5·0 % v/v sulphonated lorol	No charge	Nil
11. 0·2 % v/v sulphonated lorol	No charge	Nil
12. 1·0 % w/v cetyl pyridinium bromide	Positive	+ +
13. 0·2 % w/v cetyl pyridinium bromide	Positive	+

* + + + + + = potential of 300 V. or more ≡ 9·5 e.s.u. or more; + = potential of less than 100 V. ≡ less than 3·2 e.s.u. for a deposit on the target of approximately 2·5 g.

† Polyethylene oxide condensation products.

‡ Di-isobutylphenol poly-glycol ether.

Table 11 shows that where a charge developed on the particles it was always positive and the amount of the charge varied greatly with the solute. Two non-ionic wetting agents greatly increased the charge over that on distilled water alone, and one anionic wetting agent reduced the charge to such an extent that it could not be measured.

Most of the succeeding work was done with distilled water and solutions of Triton N 100 and Lissapol N. The latter were used because effects were more easy to measure when there was a high charge.

In order to determine if the increased charge on the target produced by some wetting agents over that given by distilled water could be due to the increased number of droplets, and to obtain some information on the potential of the individual droplets, some measurements were made of the droplet size of atomized distilled water and atomized solutions of 5 % v/v Lissapol N.

The maximum and minimum droplet size were obtained by direct measurement, and the average droplet size was obtained by a colorimetric technique. The spraying was done under the same conditions as those used for the measurement of electrostatic charges.

Direct measurement of maximum and minimum particle size. A number of methods for this purpose have been described. Recently, Hurtig & Perry (1950) have given a brief critical summary of them. The method described here was developed for this special purpose.

The bottom of the metal receiver normally used as the target was given a thin coating of vaseline, and this was covered by a layer of turpentine substitute to a depth of 2 or 3 mm. The target was then put in the apparatus and sprayed. After spraying the droplet size was measured under a binocular microscope with a calibrated micrometer eyepiece. To all appearances, droplets, both of distilled water and 5 % v/v Lissapol N remained spherical when immersed in the turpentine substitute, and tended to be held immobile by the vaseline coating until this dissolved. By this method the diameters of the droplets of distilled water were found to range from 0·016 to 0·35 mm. with a high proportion between 0·025 and 0·25 mm. The droplets of 5 % v/v Lissapol N were found to vary between 0·0125 and 0·40 mm., and again a high proportion appeared to be between 0·025 and 0·25 mm. The majority of larger droplets of the Lissapol solution appeared to contain bubbles of air.

Measurement of average particle size. Any method involving counting and measurement would appear to be exceedingly laborious and difficult.

The method adopted was to dye the solutions with aniline blue (absorption max. λ 595) and spray them on to an 8 cm. diameter metal plate placed in the metal receiver normally used as a target. The metal plate was covered to a depth of 2 or 3 mm. with turpentine substitute. A series of counts were then made under the binocular microscope of the number of droplets in a given area marked by a squared micrometer eyepiece. From these counts the total number of drops on the plate could be calculated. After the counts the total weight of spray solution on the plate was estimated by drying off the plate in a blast of hot air, and estimating the amount of dye on it colorimetrically. From these two measurements the average drop size could be calculated.

A calibration curve for each solution was prepared beforehand.

Table 12 gives the results of the measurement of the average particle size of distilled water and 5 % v/v Lissapol N.

TABLE 12. *Data on the determination by a colorimetric technique of the average droplet size of distilled water, and of* 5 % v/v *Lissapol N in distilled water, atomized under the conditions used for the determination of electrostatic charge*

(Conditions of experiment: temperature, 65–70° F.; R.H., 48–65 %; air pressure, 73 cm. Hg; 0·2 c.c. fluid in the reservoir; 5 counts of drop number for each treatment; aniline blue (absorption max. λ 595) at approximately 1° w/v in each fluid; E.E.L. colorimeter with orange filter no. 607 for colorimetric determinations.)

	5 % v/v Lissapol N in distilled water		Distilled water	
Treatment no.	Average no. of drops per 3·0625 sq.mm.	Total weight of drops on 8 cm. diameter plate (mg.)	Average no. of drops per 3·0625 sq.mm.	Total weight of drops on 8 cm. diameter plate (mg.)
1	44·2	7·5	85·4	16·0
2	65·2	6·0	87·2	20·0
3	61·8	9·5	75·4	15·9
4	79·0	10·0	65·4	14·0
5	71·6	11·2	73·0	16·0
	Mean 64·36	8·84	77·28	16·38
Total number of droplets on plate		(*a*) Distilled water		126,894
		(*b*) 5 % v/v Lissapol N in distilled water		105,679
Number of droplets per milligram		(*a*) Distilled water		7,747
		(*b*) 5 % v/v Lissapol N in distilled water		11,955
Average weight of droplet (mg.)		(*a*) Distilled water		0·00013
		(*b*) 5 % v/v Lissapol N in distilled water		0·000084
Average diameter of droplet (mm.)		(*a*) Distilled water		0·063
		(*b*) 5 % v/v Lissapol N in distilled water		0·054

These measurements indicate that the average drop size of the fluid containing the Lissapol N is about two-thirds that of the distilled water, while the range of droplet sizes is similar.

It is shown later (Table 15) that the charge per unit weight of fluid deposited varies with the pressure, but at 73 cm. Hg it appears that the charge on an average sized droplet of distilled water will be, on the average, 0·00026 e.s.u. and the potential 25 V., while the charge on the average size droplet of 5 % v/v Lissapol N will be, on the average, 0·00044 e.s.u. and the potential 49 V. Since the droplet of the atomized 5 % v/v Lissapol N is smaller, and the charge on it is higher, it would appear that the presence of the solute is altering the charge considerably, if droplets of uniform size are considered. It may be anticipated, although it has not been measured, that the droplets of atomized solutions of sulphonated lorol will be of the same order of magnitude as those of solutions of Lissapol N, and no measurable charge was found on these particles. It appears, therefore, that the presence of different wetting agents can alter, within wide limits, the charge on droplets of unit size.

Nolan & Gill (1923) have recorded the effect of a number of substances on the charging of atomized droplets, and found that those substances, giving abnormal absorption effects, had a great influence on the sign and magnitude of the charge at very low concentrations. These authors found in a number of instances a reversal of charge which did not occur in our experiment.

Two methods were used to determine if variations in electrostatic charge were likely to cause variation in deposit. The first consisted of measuring the charge for each deposit, and seeing if there were any correlation between the charge and the weight deposited. The second consists of applying a high potential to the target, and determining whether this affected the amount deposited.

Table 13 shows some of the data obtained on the relationship between amount of charge and deposit. The arrangement whereby the electrostatic measurements were taken is the same as that for the measurement of charge on different solutions which is shown diagrammatically in Text-fig. 4.

TABLE 13. *Data on the relationship between the amount of charge on the atomized droplets and the amount deposited*

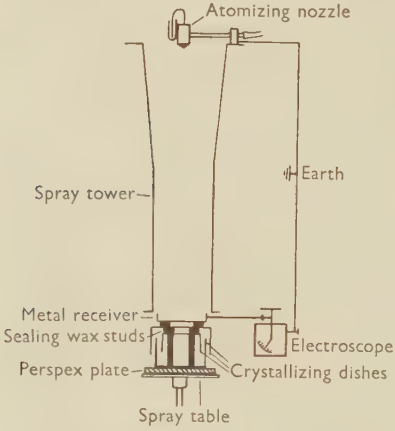
(Conditions of spraying: temperature, 64–68° F.; R.H., 55–63 %; air pressure, 72·73 cm. Hg; 10 c.c. in reservoir; spraying time, 13·0–14·5 sec.; target: circular metal receiver 9 cm. diameter with walls 1·6 cm. high; capacity of system, 10·6 pF.)

Fluid	Voltage (electroscope)	Charge (e.s.u.)	Deposit (g.)	Fluid	Voltage (electroscope)	Charge (e.s.u.)	Deposit (g.)
Distilled water	145	4·6	2·2323	5 % v/v Lissapol N	400	12·72	2·3205
	165	5·2	2·4932		375	11·92	2·2882
	150	4·8	2·4710		405	12·88	2·3341
	145	4·6	2·5605		385	12·24	2·3822
	165	5·2	2·5289		400	12·72	2·2965
0·2 % w/v cetyl pyridinium bromide	20	0·64	2·4003		365	11·61	2·4398
	25	0·79	2·5498		370	12·08	2·3248
	25	0·79	2·5648		365	11·61	2·1848
	25	0·79	2·5948		385	12·24	2·4148
	25	0·79	2·5748		415	13·20	2·4548
1·25 % w/v sulphonated lorol	0	—	2·4948	0·2 % v/v Lissapol N	155	4·93	2·5748
	0	—	2·5048		260	8·27	2·6448
	0	—	2·4798		295	9·38	2·4683
	0	—	2·3948		260	8·27	2·6298
	0	—	2·6028		295	9·38	2·5648
0·2 % w/v sulphonated lorol	0	—	2·6498		295	9·38	2·6648
	0	—	2·5198				
	0	—	2·5198				
	0	—	2·6398				

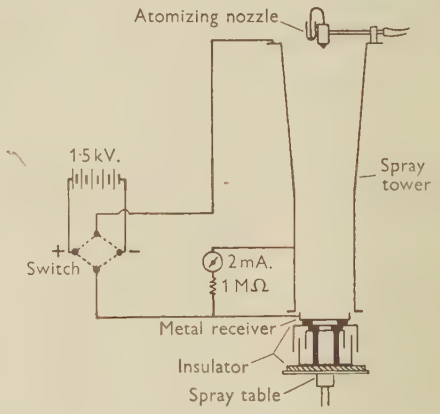
Analysis of the figures in Table 13 strongly indicates that there is no correlation between electrostatic charge on the atomized droplets and the weight of the deposit. The data for cetyl pyridinium bromide did not justify analysis since, in this instance, four out of the five charges recorded were the same. In addition, the variation occurs irrespective of the amount of the charge, for example it occurs where there

is no measurable charge, in the case of the solution of sulphonated lorol, as well as in the case of the solution of Lissapol N where there is a high charge.

In order to determine whether a charge on the target would affect the amount of the deposit on it, potentials from 100–700 V. were first tried with no measurable effect. Finally an applied potential of 1500 V. was tested, still without any measurable effect on the weight of the deposit. Text-fig. 5 shows diagrammatically the arrangement of the apparatus for this measurement, and Table 14 gives a sample of the figures obtained using distilled water and two spraying pressures. An analysis of variance showed that there was no significant difference between the deposit obtained with the positive charge on the plate and that obtained with the negative charge. This holds true for both spraying pressures.



Text-fig. 4.



Text-fig. 5.

Text-fig. 4. Diagram of arrangement for measuring the charge on atomized droplets reaching the spray target.

Text-fig. 5. Diagram of the arrangement for testing the effect of potential applied to the spray target on the weight of the deposit.

The overall evidence thus strongly indicates that under the spraying conditions obtaining in the tower where the atomized droplets are impacted on the target by a stream of air, large differences of electrostatic charge on the droplets or the spray target will not affect the weight of the deposit, and are therefore not likely to be a source of variation of the deposit.

In order to decide how far the conditions of electrification examined might be regarded as representative, some further observations were made on the factors influencing the amount of electrostatic charge on the droplets. Table 15 shows that the amount of charge per unit weight of deposit depends on the pressure of atomization, and Table 16 shows that the amount of charge on the target at any given pressure varies with the amount of the deposit.

TABLE 14. *Effect of applied electrostatic charge on the target on the weight of deposit of atomized droplets of distilled water*

(Conditions of the experiment: temperature, 63–64° F.; R.H., 63–67 %; 10 c.c. of liquid in the reservoir; target: a circular metal receiver, 9 cm. diameter with walls 1·6 cm. high, placed on an insulator.)

Air pressure = 73 cm. Hg		Air pressure = 18 cm. Hg	
Potential of target	Deposit (g.)	Potential of target	Deposit (g.)
1·5 kV.+	2·5522	1·5 kV.+	2·3370
1·5 kV.−	2·5716	1·5 kV.−	2·3490
1·5 kV.+	2·5300	1·5 kV.+	2·4670
1·5 kV.−	2·5533	1·5 kV.−	2·4473
1·5 kV.+	2·5583	1·5 kV.+	2·4420
1·5 kV.−	2·5853	1·5 kV.−	2·4470
1·5 kV.+	2·5745	1·5 kV.+	2·4970
1·5 kV.−	2·5894	1·5 kV.−	2·4570
1·5 kV.+	2·5703	1·5 kV.+	2·4120
1·5 kV.−	2·5339	1·5 kV.−	2·4720

TABLE 15. *Variation in electrostatic charge with pressure of atomization using* 5 % v/v *Lissapol N in distilled water*

(Conditions of the experiment: temperature, 66° F.; R.H., 60 %; 10 c.c. of liquid in the reservoir; target: a circular metal receiver, 9 cm. diameter with walls 1·6 cm. high, placed on a insulator.)

Pressure (cm. Hg)	Weight of deposit (g.)	Voltage (electro-scope)	Charge (e.s.u.)	Pressure (cm. Hg)	Weight of deposit (g.)	Voltage (electro-scope)	Charge (e.s.u.)
118·5	2·1460	560	17·81	86	2·1580	425	13·51
118·5	2·3030	600	19·08	73	2·3080	360	11·45
118·5	2·3350	600	19·08	73	2·3600	410	13·04
118·5	2·2880	525	16·69	73	2·3100	365	11·60
102	2·1280	460	14·63	38	2·4950	245	7·79
102	2·1530	435	14·03	38	2·5880	245	7·79
102	2·2250	420	13·36	38	2·6150	255	8·11
86	2·2700	410	13·04	18	2·6320	125	3·97
86	2·1950	360	11·45	18	2·5980	125	3·97
86	2·3150	360	11·45	18	2·7000	135	4·29
86	2·2530	445	14·15				

TABLE 16. *Variation in electrostatic charge on the target with amount sprayed from the reservoir, using* 5 % v/v *Triton N* 100 *in distilled water*

(Conditions of the experiment: temperature, 66° F.; R.H., 60 %; air pressure, 118·5 cm. Hg; target: a circular metal receiver, 9 cm. diameter with walls 1·6 cm. high, placed on an insulator. Capacity of the system, 10·6 pF.)

Amount in reservoir (c.c.)	Time to spray (sec.)	Voltage (electroscope)	Charge (e.s.u.)
5	9·5	540	17·2
4	6	440	14·0
3	5	335	10·7
2	3·5	235	7·5
1	1·25	100	3·2

Since the evidence suggests that differences in electrostatic charge on the droplets are unlikely to be responsible for the observed variations in deposit, some consideration may be given to turbulence as a possible factor causing variation.

(*c*) *Turbulence*

In the apparatus described, turbulence occurs in the spray tower and it is believed that, by breaking up the cone of spray issuing from the atomizing nozzle, it helps to produce even distribution over the spray plate.

It seems, however, that if an air stream is directed down a tube a number of times in such a manner that turbulence is produced, the point in the tube where turbulence begins and hence, in a given tube length, the total amount of turbulence, may vary, and cannot easily be stabilized. The amount of turbulence affects the amount of spray fluid thrown out on the walls of the tube and hence the amount reaching the spray target.

No useful experimental data on the amount of turbulence occurring from one spraying to the next have been obtained with this apparatus, but it would appear to be the most likely explanation of the variations that occur in the replication of the deposit over a series of weighings, in the light of the data showing that environmental, and electrostatic effects are not important.

It does not, however, appear to provide an explanation of the drift in average weight that occurs over a period of time. This indicates that there are sources of variation which have not yet been recognized. It is essential, therefore, that the weight of the deposit should be checked at frequent intervals.

Discussion and conclusions

It is difficult to make a fair comparison between the physical performance of this apparatus and others described in the literature, owing to the number of factors that have to be considered, particularly those outlined above which may cause variations in the deposit. Perhaps it may be said that there is no other apparatus described which may be used to apply an equal replicable dose of insecticide as a direct spray to all individuals of adequately large batches of a wide variety of species of insect, which may also be used to apply even residual films on a wide variety of surfaces of adequate area, and at the same time may be used with media varying from distilled water to a heavy petroleum oil.

Biological performance

No details of biological performance are given in this paper, but a considerable amount of data on insecticidal action which have been obtained with the apparatus described or minor modifications of it have been published. Two recent publications giving such data are Elliott, Needham & Potter (1950) and Lord & Potter (1951). In addition to the information given at the same time as the apparatus was first described in 1941, some information on the factors in the technique affecting

the biological effect of poisons applied in petroleum oil media by means of earlier modifications of this apparatus has been given by Tattersfield & Potter (1943) and Parkin & Green (1943), and a study of the factors in the technique affecting the toxicity of aqueous sprays applied both directly and as a residual film is at present in progress.

I am greatly indebted to Dr Frank L. Howard of the University of Rhode Island for the invitation to work in his department, and for providing facilities during my stay, and to Prof. E. Schock of the engineering department of the University for help in the design and manufacture of the intermediate form of nozzle. I am also greatly indebted to Mr A. J. Arnold of the Insecticides Department of Rothamsted for a great deal of technical help, and to Dr W. C. A. Hutchinson of the Physics Department of Rothamsted for guidance in the work on electrostatic charge.

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EXPLANATION OF PLATE 1

Fig. 1. General view of intermediate design of apparatus.

Fig. 2. General view of final design of apparatus.

Fig. 3. Top of spray tower, intermediate design of apparatus, showing the atomizing nozzle and the atomizing nozzle mounting.

Fig. 4. Top of spray tower, final design of apparatus, showing the atomizing nozzle and the atomizing nozzle mounting.

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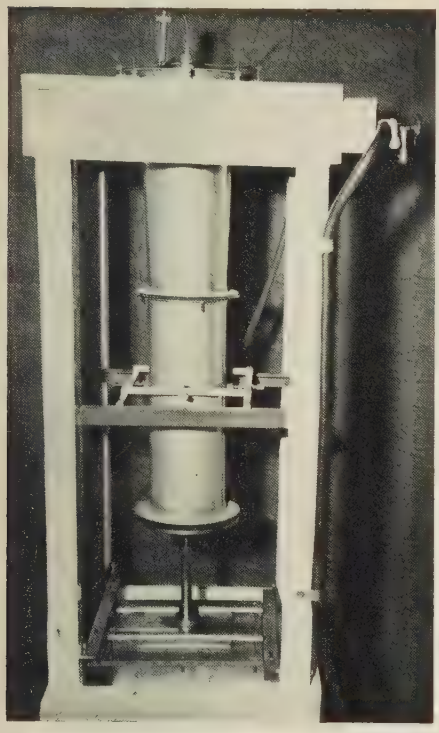

Fig. 1.

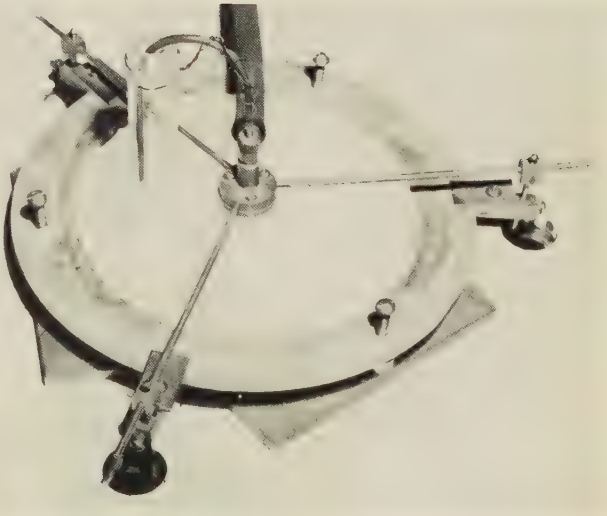

Fig. 3.

Fig. 2.

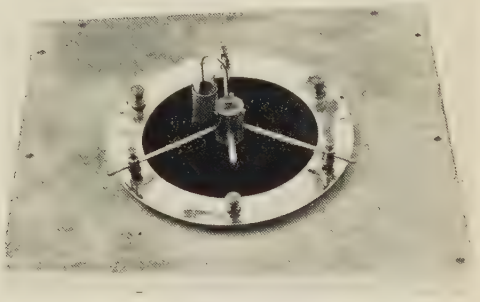

Fig. 4.

DRY-ROT DISEASE OF THE POTATO*

I. EFFECT OF COMMERCIAL HANDLING METHODS ON THE INCIDENCE OF THE DISEASE

By C. E. FOISTER,† A. R. WILSON‡ and A. E. W. BOYD§

A study has been made of the incidence of dry rot in seed potatoes in relation to commercial handling methods, and it has been shown that infection following grading by mechanical reciprocating riddles fitted with bare wire screens is more serious than that following normal handling at harvest or during transport. Hand picking, hand riddling and the use of rubber-spool graders or rubber-coated screens reduced infection by varying amounts. The higher incidence of the disease in 'transported' as against 'home-saved' seed is regarded as being due to machine grading of such stocks some months prior to planting.

Contamination of stores or boxes has not been found to play any material part in the spread of infection under commercial conditions unless tubers are roughly handled during storage. As contact infection has been found to be rare, it is considered that the practice of 'picking over' stocks during the winter to remove diseased tubers is unnecessary and may lead to further infection.

In addition to wounds, lesions of both blight and powdery scab, but not common scab, have been found to be a means of infection.

Although dry rot may sometimes cause considerable losses in seed potatoes of susceptible varieties retained on the farm of origin, the disease is usually more serious in 'transported' stocks such as those received in England from Scotland and Ireland or those sent from one area of England to another. It has been established that dry rot is a soil-borne disease, and that the principal causal agent, *Fusarium caeruleum* (Lib.) Sacc., is present in many field soils in Britain (Small, 1944, 1945; Foister, Wilson & Boyd, 1945). Observations made by the writers indicate that infestation is in fact widespread wherever susceptible varieties are grown.|| This suggests that the factor responsible for the greater extent of infection in 'transported' seed lies in differences in the methods of handling, and evidence that this is so was obtained during the course of this investigation. Some of the work described has already been summarized in papers by Foister & Wilson (1943) and Boyd (1947).

* This investigation of various problems associated with the dry-rot disease of potatoes was begun in 1942 under the joint auspices of the Agricultural Research Council and the Department of Agriculture for Scotland.

† Department of Agriculture for Scotland, Plant Pathology Laboratory, East Craigs, Corstorphine, Edinburgh.

‡ Agricultural Research Council's Potato Storage Investigation, c/o Nottingham University School of Agriculture, Sutton Bonington, Loughborough, Leics.

§ Edinburgh and East of Scotland College of Agriculture, George Square, Edinburgh (until 1946 with the Agricultural Research Council).

|| The investigations on soil infestation will be reported in a subsequent paper.

Materials and methods

Stocks of the susceptible varieties Doon Star and Arran Pilot grown in Scotland (where possible on fields known to be infested) were used. As random sampling from the growing crop is impracticable on a large scale under commercial conditions, where replicated trials were required, bags were filled from one clamp and allocated at random to the treatments. Replicates were carried out on lots of not less than 56 lb., treatments frequently involved 2–5 cwt., while normally more than 1 ton was used for each experiment. Experimental consignments sent to England were stored in a sprouting house at the University of Nottingham School of Agriculture. When required, a randomized layout was employed for box and bag storage to eliminate positional effects. Throughout the experimental work care was taken to follow as closely as possible normal commercial practice; any variation from this was made to ascertain the importance of some specific factor and is duly noted. Grading was by power-operated reciprocating riddles fitted with bare wire screens, of the type commonly used on Scottish farms, unless otherwise stated.

Certain of the data presented are abstracted from the results of larger trials including chemical treatments for the control of the disease, which are outside the scope of the present paper.

Time and method of infection

The results of numerous experiments carried out by the writers support the view advanced by Small (1944, 1945, 1946) that wound infection accounts for all but a negligible proportion of the disease under commercial conditions. Various experiments (McKee & Boyd, 1952) have also confirmed the findings of Small (1945) and Lansade (1949) that *F. caeruleum* remains viable in dry soil, either loose or adherent to tubers, for periods of over one year, and that contaminated tubers can be infected through wounds made at any time during storage life. Pethybridge & Lafferty (1917) showed that tubers exhibited seasonal variation in susceptibility to dry-rot infection. These authors indicated that in early autumn tubers were relatively resistant and that susceptibility increased throughout the storage period, reaching a maximum in early spring. The findings of Small (1945), Lansade (1949), and those of the writers, are in general agreement with this. These facts, in conjunction with the widespread infestation of soils in potato-growing districts noted previously, indicate the importance of both time and extent of damage sustained during handling.

Under commercial conditions in Britain there are normally four main times at which seed tubers are exposed to mechanical injury: (1) on harvesting, (2) on grading, (3) during transport, and (4) during storage when tubers are boxed. The standard practice in Scotland is to lift the crop at some time during August, September or October, depending upon the variety. Although some grading and despatch may be done immediately, it is usual to clamp the crop on the field.

Grading, bagging and despatch are then carried out from late October onwards. On receipt in England, the seed, especially that of early varieties, is frequently boxed to sprout until required for planting. During this time it is common practice to 'pick over' stocks, particularly those severely affected with dry rot, in order to remove diseased tubers.

Infection following harvest

Potatoes are usually lifted by digger plough, elevator digger or spinner. In general, when correctly used, the plough causes the least damage and the spinner the most, but no satisfactory figures have been obtained to demonstrate the comparative effect of these methods of lifting on dry-rot infection. Damage may also be caused during harvest by throwing potatoes into the carts, by walking on the loads and by the forks used to trim the clamps.

It is commonly thought that the bulk of infection is associated with damage caused at harvest time; this belief has been strengthened by the fact that control methods, such as dipping, are usually most effective when applied immediately after lifting. In four experiments carried out in 1943, however, it was found that infection at this time was relatively slight (Table 1); subsequent experience has shown that figures of the order given are representative of the normal development of the disease in clamp in Scotland.

TABLE 1. *Amount and time of development of dry rot in Scottish seed potatoes sent to England*

(Tubers clamped on lifting; on removal from clamp riddled, bagged and despatched; on receipt boxed. Season 1943–4.)

		On removal from clamp in Scotland		During storage in England. No. of tubers with dry rot in 2 cwt. 'seed'		
Variety	Date lifted and clamped	Date	No. of tubers with dry rot in 6 cwt. 'as grown'*	Dec.–Feb.	Feb.–Apr.	Total
Doon Star	4 Oct.	10 Dec.	5	61	84	145
		24 Feb.	0	—	86	86
Doon Star	30 Sept.	8 Dec.	0	129	328	457
		22 Feb.	15	—	802†	802
Arran Pilot	23 Sept.	7 Dec.	0	83	122	205
Arran Pilot	4 Aug.	9 Dec.	23‡	572	97	669
		24 Feb.	0	—	159	159

* 'As grown' denotes the crop before size grading.
† Presuming 500 tubers/cwt., 802 tubers in 2 cwt. = 80·2 %.
‡ Presuming 400 tubers/cwt., 23 tubers in 6 cwt. = 0·96 %.

What may be regarded as infection following damage at lifting time is shown in Table 1, column 4. Differences between the December figures and those obtained when removing further tubers from the same clamps in February are

small and may be ascribed largely to sampling error, thus indicating that the bulk of infection which took place at harvest time had in fact become visible by December. The reason for the relatively low level of infection following lifting may be due either to a low incidence of damage to the tubers at that time (particularly as to the proportion of skin area broken), to greater resistance of the tubers in autumn, or to a combination of the two factors. That clamp environment is not responsible is indicated by the severe dry rot which may develop in ware tubers re-clamped during the winter after the seed has been riddled out.

Infection following grading

Grading is normally carried out directly from the clamp on power-operated, reciprocating riddles with bare wire screens or on hand riddles. The seed is then bagged and despatched. Apart from some gross injury caused during forking on to the elevator of the riddle, damage to the tubers during machine grading is confined mainly to skin abrasions caused by the rapid shaking to and fro on the screens. Under normal conditions each tuber is subjected to this shaking movement some fifty times. These abrasions are often very numerous, although almost invisible, and may involve a much higher proportion of the skin area than the wounds sustained at harvest time, thus greatly increasing the chance of infection.

The figures for infection occurring between grading and planting shown in Table 1, column 7, are an indication of the seriousness of post-harvest damage most of which, as will become evident, is attributable to injury during grading. In Table 2 are given the results of an experiment in which tubers were graded in four different ways: (1) twice by a mechanical riddle of the reciprocating type, fitted with a bare wire screen (to simulate a possible regrading on receipt), (2) once by the same machine, (3) by passing tubers over an inclined slatted 'grading table' down which they could slide easily so that ware and rotten potatoes could be removed by hand, and (4) by hand directly from the clamp face. Subtreatments following grading were designed to provide data on the comparative effects of normal and rough handling during subsequent transport and will be discussed in a later section.

Table 2. *Development of dry rot in tubers of the variety Arran Pilot graded in four different ways*

(Tubers lifted and clamped in October, graded and despatched to England (by road) in December. Season 1946–7.)

Method of grading	Percentage dry rot (April)	
	Bags handled normally	Bags handled roughly
Mechanical reciprocating riddle (bare wire screen)—twice	24	42
Mechanical reciprocating riddle (bare wire screen)—once	16	26
'Grading table'	3	7
Hand-picked	4	14

The results of a further experiment are given in Table 3. Here the effects of five methods of grading on the development of dry rot are compared.

TABLE 3. *Development of dry rot in tubers of the variety Arran Pilot graded by five different methods*

(Tubers graded on lifting in October, retained at farm of origin in Scotland, bagged for 14 days, then boxed. Season 1947–8.)

Method of grading	Percentage dry rot (March)
A. Mechanical reciprocating riddle (bare wire screen)	9·4
B. Mechanical reciprocating riddle (rubber-coated screen)	4·0
C. American rubber-spool grader	3·5
D. Hand riddle (bare wire screen)	2·3
E. Hand-picked	1·5

Significant differences between treatment effects ($P < 0{\cdot}05$), BCDE < A, E < BC.

The figures in Tables 2 and 3 show that the use of a bare wire screen on the mechanical reciprocating riddle led to serious infection. The results given in Table 3 show that the risk of infection may be materially reduced by the use of rubber-coated screens. The American rubber-spool grader also showed promise in this direction, but no machine of this type is manufactured in Britain. The small amount of dry rot that developed in hand-riddled tubers is interesting, and suggests that considerably less damage was caused than by the bare wire screen on the mechanical riddle. This is presumably because agitation on the hand riddle is less violent and for a shorter period of time. Tubers 'table-graded' in the first experiment (Table 2) and hand-picked in both experiments (Tables 2 and 3) were little damaged and developed only a small amount of dry rot.

Although it is impossible in the experiments detailed in Tables 2 and 3 to isolate infection resulting from grading damage from that associated with injury received during transport and subsequent storage, as the periods of damage follow closely upon each other, the low level of infection in the hand-picked, normally handled tubers, shown in Table 2, suggests that in this experiment, damage sustained through normal handling subsequent to grading was not of great importance. A similar conclusion is reached from consideration of the figures in Table 4 (in the experiment shown in Table 3 the transport factor is eliminated). In connexion with the results discussed above, it is interesting to note that Mooi (1950), working in Holland, found that infection following machine grading rarely exceeded three times that occurring after hand picking (cf. Tables 2 and 3 above). Mooi found that the greatest increase in infection followed the use of 'shock-system' graders.

Infection following transport

Transport of seed potatoes from Scotland, particularly of early varieties, is usually by road or rail. Damage during transport is caused mainly by rough handling of the sacks and consists either of friction abrasions, bruises or gross

splitting. The 'rough' handling treatment shown in Table 2 consisted of dropping the bags from shoulder height on to a hard road and walking on them several times. It should be stressed that this degree of maltreatment is most unlikely to occur in practice, except possibly in the case of a few bags in a consignment; comparison of the figures for infection following normal and rough handling show, however, how serious the consequences of such maltreatment can be. Road transport involves handling the bags on and off one vehicle. Rail transport involves handling the bags on and off a lorry at each end as well as on and off the railway wagon. For this reason tubers transported by rail might be expected to be more liable to infection than those transported by road. No comparative figures are, however, available. Figures in Table 4 show that tubers transported by road to England and left in bags developed 14 and 6 % dry rot compared with 8 and 1 % which developed in those retained in bags at the farm of origin. This is the only pair of treatments where the effect of transport alone can be assessed.

Table 4. *Effect of road transport and environment on development of dry rot in tubers of the variety Arran Pilot*

(Tubers lifted in October and clamped, other treatments in December. Season 1946–7.)

Treatment	Percentage dry rot (April)	
	Machine graded	Hand-picked
Bagged, despatched to England, and left in bags	14	6
Bagged, despatched to England, and boxed	16	4
Retained at farm, bagged	8	1
Retained at farm, boxed	4	4

In general, the figures show that transport causes a more marked increase in infection of severely damaged (machine-graded) tubers than it does of comparatively undamaged (hand-picked) tubers. Such a secondary action could be due to the humid environment of the sacks immediately following grading, but a comparison of the figures in Table 4, showing the extent of infection in boxed and bagged tubers retained on the farm, does not entirely support this explanation. Support is, however, given by two experiments in which, following grading, clamped tubers developed 15 and 20 % dry rot, whereas boxed tubers developed 7 % only in both instances. In one experiment also, wetting tubers during grading prior to transport in bags raised infection from 16 to 23 %. Previously published evidence on the influence of relative humidity on infection is conflicting (Small, 1944, 1946), and final proof must be provided by studies under conditions of controlled environment.

Infection during storage in boxes

Store contamination. Pethybridge & Bowers (1908), Small (1945) and Lansade (1949) showed that *F. caeruleum* is often present in the air in potato stores. Small also showed that the fungus may remain viable in dust for long periods, but

concluded that unless tubers were freshly damaged after a period of storage the risk of infection was negligible. This view is supported by the evidence of one experiment carried out by the writers in which 2 cwt. of each of two stocks of Arran Pilot were riddled and boxed on lifting in October, then divided equally between a heavily contaminated and a clean store in Scotland. In neither case were the tubers damaged in any way other than that incidental to normal handling. In the contaminated store 0·5 and 2·0 % dry rot had developed by April against 0·3 and 3·7 % in the clean store.

Box contamination. Pethybridge (1917) mentions box contamination as a source of infection. Small (1944) demonstrated by experiment that danger of infection from this source existed only when tubers were freshly damaged, and concluded that under normal commercial conditions the risk was negligible. Experimental evidence obtained by the writers supports this view. Figures from a representative experiment are given in Table 5.

TABLE 5. *Effect of box contamination on bruised and normally handled tubers of the variety Doon Star*

(Season 1945–6.)

	Percentage dry rot (April)	
Treatment	Sterilized boxes	Contaminated boxes
Bruised	5	29
Normally handled	0	2

In this experiment six boxes of 100 tubers were used for each subtreatment. Sterilized boxes had been dipped in 5 % formalin and stored out of doors until required. Contaminated boxes had been used for storing diseased tubers for 10 weeks during the previous season, and had then been retained in store until required. 'Bruised' tubers were dropped on sharp sterilized stones. While it is admitted that both contaminated stores and boxes may increase the available inoculum on the tubers, this would appear, in view of the apparent prevalence of soil infestation in Britain and consequent contamination of the tubers, to be immaterial under any circumstances. If, on the other hand, tubers have been disinfected (Small, 1945) or soils are relatively free from infestation (Lansade, 1949), store and box contamination might prove a factor of some importance if winter handling of the stocks in store was necessary.

Contact infection. Small (1944) reported that when tubers were bruised contact infection was considerable, but when they were undamaged it was uncommon. From this he concluded that this method of infection was not of any importance commercially provided the potatoes were left undisturbed. During the examination of many hundreds of diseased stocks in storage, the writers have observed contact infection of unwounded tubers in a few instances only, and in these there was evidence of a local poisoning effect due to the decomposition of the adjacent

diseased tuber. Attempts to induce contact infection of unwounded tubers in sprouting boxes experimentally failed. Any 'picking over', except that required shortly before planting is, therefore, unnecessary. As heavy contamination and at least some injury to the tubers is unavoidable during the process, the practice may in fact lead to further infection.

Other means of infection

Pethybridge & Lafferty (1917) mention that *F. caeruleum* can infect tubers through scab lesions, the type of scab being unspecified. An experiment carried out on lesions of common scab (*Streptomyces scabies*) using a spore suspension of *Fusarium caeruleum* gave no indication that penetration through such lesions took place. On the other hand, penetration through lesions of powdery scab (*Spongospora subterranea*) has frequently been observed, although in comparison with mechanical damage as a means of penetration it is usually of minor importance. In one experiment, in 1942, counts made on 155 diseased tubers from a stock of Arran Pilot showed that 77 % of the dry-rot lesions originated from, or were associated with, pustules of powdery scab.

Dry rot is frequently found as a secondary infection in tubers affected with blight (*Phytophthora infestans*). It is interesting to note that during the course of the investigation dry-rot lesions have not been found in association with growth cracks or damage caused by wireworms or slugs.

Discussion

The present work, which was done concurrently with that of Small (1944, 1945, 1946), has confirmed his findings relating to the source, method and time of infection. In addition, it has demonstrated that under normal commercial conditions in Britain, the damage to the tubers caused by grading on the bare wire screen reciprocating riddle, which is commonly used, influences infection to a far greater extent than that caused by harvesting or transport or any other form of handling. 'Transported' seed must be graded prior to despatch from the farm of origin and for this reason riddling often takes place a considerable time before planting. The seed is usually despatched very soon after riddling, in sacks where the potatoes are normally under relatively humid conditions. In 'home-saved' seed grading is often done by hand after harvest, in which case little damage is caused and infection is usually further discouraged by subsequent storage in boxes in a reasonably dry environment. Alternatively, grading may be postponed until immediately prior to planting, in which case, even if a machine is used, the disease has insufficient time in which to develop. Where serious dry rot occurs in 'home-saved' seed it can often be traced to abnormal damage caused, for example, by excessive handling after picking, or by machine grading at or soon after lifting or during the winter (Small, 1945). The above considerations seem quite sufficient

to explain the greater incidence of dry rot in 'transported' as compared with 'home-saved' seed.

One may conclude that the most important measure in the control of dry rot is to minimize damage during grading. Hand picking or 'table grading' are to be preferred to machine grading; indeed on many small farms 'home-saved' seed is hand-picked and 'table grading' is used commercially in both New Zealand and Ireland* by some growers of high-quality seed. If neither of these methods can be adopted, the use of rubber-coated screens on reciprocating graders is advocated. Hand riddles have also been shown to cause less damage than the mechanical reciprocating type with bare wire screens. Chemical treatment should only be necessary when such precautions are impossible, or as an additional safeguard where heavy soil infestation is present or suspected.†

The authors are indebted to Mr G. Samuel for his valuable advice and criticism during the preparation of the manuscript. They also gratefully acknowledge the co-operation of numerous Scottish seed-potato growers and merchants.

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* Details of a 'grading table' are published by the government of Northern Ireland, Ministry of Agriculture, in a leaflet entitled 'A potato chute for the farm'. (*Talks to producers*, no. 40.)

† A later paper in the present series will deal with experiments on the control of dry rot by chemical treatment of the tubers.

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DRY-ROT DISEASE OF THE POTATO

II. FUNGI CAUSING DRY ROT OF SEED POTATOES IN BRITAIN

By R. K. McKEE

Agricultural Research Council, Potato Storage Investigation, c/o University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leicestershire

Examination of 144 samples of seed potatoes affected with dry rot, comprising 1218 tubers, showed the presence of parasitic Fusaria in 91 % of the lesions. The parasitic Fusaria consisted of 93 % *Fusarium caeruleum*, 6 % *F. avenaceum* and less than 1 % each of *F. arthrosporioides* and *F. tricinctum*. *F. tricinctum* has not previously been recorded on potato in this country.

A method is described whereby the presence of *F. caeruleum* can readily be detected in tubers affected with dry rot.

Many species of *Fusarium* have been isolated from potato tubers (Appel & Wollenweber, 1910; Sherbakoff, 1915); a number of these have proved to be troublesome parasites in other countries, but for many years the only one recorded as causing appreciable damage to stored potatoes in Britain was *F. caeruleum* (Lib.) Sacc. Recently, however, Moore (1945) isolated *F. avenaceum* (Fr.) Sacc. from rotted tubers collected during a survey of the causes of wastage in potato clamps in England, and confirmed its pathogenicity by artificial inoculation; *F. avenaceum* is widely distributed in Great Britain and is often responsible for foot-rot of cereals.

In experiments in which a biological method of detecting tuber pathogens in field soils was used (McKee & Boyd, 1952), isolations were made from a large number of the lesions which developed on the inoculated tubers. *F. caeruleum* was found to be responsible for the majority of these lesions, though a considerable proportion of the actively spreading rots was caused by *F. avenaceum*; two other Fusaria, *F. tricinctum* (Corda) Sacc. and *F. arthrosporioides* Sherb.,* were found only occasionally. *F. tricinctum* has not previously been recorded as a tuber parasite in this country, but *F. arthrosporioides* has been reported from Ireland (Pethybridge & Lafferty, 1917). The pathogenicity of these four Fusaria in pure culture was confirmed under laboratory conditions.

As the tuber rots caused by these fungi are similar in external appearance it was considered desirable to investigate the relative importance of the four fungi as causal agents of dry rot of seed potatoes under natural conditions. For this purpose samples of tubers affected with dry rot were collected, in the spring of 1949 and 1950, from seed stored in sprouting houses in Lincolnshire. A number of samples

* Prof. C. D. Sherbakoff, The University of Tennessee, Agric. Exp. Sta., Knoxville 16, kindly identified cultures of these fungi. *F. arthrosporioides* is closely related to *F. avenaceum* or may be merely a variant of that species, but *F. tricinctum* is distinct, although the appearance in culture of all three is very similar.

were also collected, during 1948 and 1949, by Plant Pathologists of the National Agricultural Advisory Service, members of the National Association of Seed Potato Merchants and others. In all, 144 samples, comprising 1405 tubers, were examined; fifty-one of them were collected in the 1949 Lincolnshire survey, forty-one in the 1950 Lincolnshire survey, while the remaining fifty-two were obtained from the miscellaneous sources mentioned. Some of the tubers were found to be damaged by frost or affected with blight, but the great majority (1218) showed typical dry-rot lesions.

Although most of the samples were of the varieties Arran Pilot, Majestic or King Edward (those most commonly grown in Lincolnshire), many other varieties were encountered in smaller numbers. The samples included both Irish, Scotch and 'home-saved' seed.

Methods

Collection of samples

Each sample consisted of ten tubers affected with dry rot, provided this number could be collected from the stock under examination. Only one sample was taken from each stock (i.e. each lot of potatoes covered by the same certificate number), except in a few instances where the same stock was found in more than one sprouting house. All the stocks in the sprouting houses visited were examined, no selection of stocks being made.

Examination of samples

It is sometimes possible to determine the causal fungus in specimens of dry rot from the presence of characteristic sporodochia on the lesions or, in certain varieties, even from the colour of the rotted flesh (McKee & Boyd, 1952), but generally the fungus must be examined in culture before a definite identification can be made. Isolation by plating presents no difficulty but is laborious when large numbers of samples have to be examined. After the first season's work, a modified method was adopted when it was found that sporulation occurred within a few days on small pieces of rotted tissue kept at a high humidity (on moist filter-paper in Petri dishes). Under these conditions *F. caeruleum* soon formed its characteristic spores; *F. avenaceum*, *F. arthrosporioides* and *F. tricinctum*, however, produced a variety of spore types that were mutually indistinguishable, though recognizable as a group which could not be confused with *F. caeruleum*. Although specific identification of Fusaria other than *F. caeruleum* could be made only from mature cultures, the number of isolations necessary was greatly reduced by elimination of the *F. caeruleum* lesions in the way described. *Phoma* and *Cylindrocarpon* spp., when present, were easily recognized but were not identified specifically. As a check, a considerable number of lesions were classified by both methods, using adjacent pieces of rotted tissue in each instance. Group separation by identification of the spores produced on tissue fragments showed good agreement with the results of isolation.

TABLE 1. *Results of examination of samples of tubers affected with dry rot*

Variety	No. of samples	No. of tubers	Parasitic Fusaria isolated (%)	Secondary organisms isolated (%)	Bacteria or no growth (%)	Relative frequency of isolation of parasitic Fusaria: *F. caeruleum* (%)	*F. avenaceum* (%)	*F. arthrosporioides* (%)	*F. tricinctum* (%)
Arran Pilot:									
Misc. samples	18*	203	96·1	1·0	2·9	95·9	3·6	0·0	0·5
1949 survey	14	113	93·8	1·8	4·4	96·3	3·7	0·0	0·0
1950 survey	8	79	100·0	0·0	0·0	98·8	1·2	0·0	0·0
All samples	40	395	96·2	1·0	2·8	96·6	3·1	0·0	0·3
Doon Star:									
Misc. samples	2	20	95·0	0·0	5·0	79·0	21·0†	0·0	0·0
1949 survey	4	40	100·0	0·0	0·0	97·6	2·4	0·0	0·0
1950 survey	3	28	96·4	0·0	3·6	92·6	7·4	0·0	0·0
All samples	9	88	97·8	0·0	2·2	92·0	8·0	0·0	0·0
Home Guard:									
Misc. samples	2	8	50·0	12·5	37·5	75·0	25·0	0·0	0·0
1949 survey	1	8	75·0	12·5	12·5	83·3	16·7	0·0	0·0
1950 survey	4	25	44·0	20·0	36·0	90·9	9·1	0·0	0·0
All samples	7	41	51·2	17·1	31·7	85·7	14·3	0·0	0·0
King Edward:									
Misc. samples	1	1	100·0	0·0	0·0	0·0	100·0	0·0	0·0
1949 survey	8	59	91·5	8·5	0·0	63·0	27·7	5·6	3·7
1950 survey	7	49	89·8	2·0	8·2	68·1	27·3	2·3	2·3
All samples	16	109	90·7	5·5	3·8	64·7	28·3	4·0	3·0
Majestic:									
Misc. samples:	9	72	100·0	0·0	0·0	100·0	0·0	0·0	0·0
1949 survey	11	108	99·1	0·9	0·0	98·1	1·9	0·0	0·0
1950 survey	13	109	89·0	3·7	7·3	92·8	7·2	0·0	0·0
All samples	33	289	95·5	1·7	2·8	96·7	3·3	0·0	0·0
Other varieties:									
Misc. samples	20	151	81·6	9·2	9·2	94·4	3·2	0·0	2·4
1949 survey	13	101	84·3	13·7	2·0	95·3	1·2	1·2	2·3
1950 survey	6	44	84·1	0·0	15·9	86·5	8·1	2·7	2·7
All samples	39	296	82·9	9·4	7·7	93·6	3·2	0·8	2·4
All varieties:									
Misc. samples	52	455	91·1	3·7	5·2	94·9	4·1	0·0	1·0
1949 survey	51	429	92·9	5·3	1·8	92·0	6·0	1·0	1·0
1950 survey	41	334	88·3	3·0	8·7	89·8	8·8	0·7	0·7
All samples	144	1218	90·9	4·1	5·0	92·6	6·0	0·5	0·9
Blighted tubers‡		187	44·0	30·9	25·1	53·6	38·0	3·6	4·8

* Some of these samples comprised more than 10 tubers. † All in one sample. ‡ See text.

Results

The results are summarized in Table 1. The data for the most common varieties are set out separately, those varieties of which few samples were examined being grouped under 'Other varieties'. Each variety is subdivided so that the results from the 1949 and 1950 Lincolnshire surveys are given separately, while those from the remaining samples obtained from various sources in 1948 and 1949 are combined under 'Miscellaneous samples'. Appropriate totals are given to facilitate comparison of the effects of variety and season. Table 2 shows the relative frequencies with which the species included under 'Secondary organisms' in Table 1 were isolated; here the results for all the varieties and seasons are combined.

Table 2. *Relative frequency of isolation of fungus species included under 'Secondary organisms' in Table 1*

	F. culmorum (%)	*F. sambucinum* (%)	*Cylindrocarpon* sp. (%)	*Phoma* spp. (%)
All varieties, all samples	16·0	2·0	44·0	36·0
Blighted tubers*	8·5	3·4	72·8	15·3

* See text.

Parasitic Fusaria were found in 91% of all the dry-rot lesions examined, and consisted of 93% *F. caeruleum*, 6% *F. avenaceum* and less than 1% each of *F. arthrosporioides* and *F. tricinctum*. The fungi included under 'Secondary organisms' were found in 4% of the lesions; they are species usually considered to be saprophytic on potato tubers and are often present in old lesions. Their pathogenicity has not been checked in this work except in the case of *F. culmorum*, twenty isolates of which were found to be non-pathogenic except perhaps in association with soft-rotting bacteria. Tubers affected by gangrene (*Phoma foveata* Foister) were not collected, and it is thought that the *Phoma* spp. recorded were probably saprophytes. Although isolations were not made from lesions in which soft-rotting bacteria were obviously active, bacteria were usually present to some extent and were probably responsible for failure to isolate any fungus from many of the lesions included in the category 'Bacteria or no growth' (5%).

A number of tubers apparently affected by dry rot were found, on cutting, to be affected by blight. Although these tubers were not included in the main survey they were examined in the same way as those affected by dry rot only; the relative frequencies of the species of fungi isolated are given at the end of Tables 1 and 2. *F. caeruleum* is often present in such tubers but other *Fusarium* species are proportionately much more frequent than in tubers not affected by blight. A species of *Cylindrocarpon* was also more frequently associated with the blighted tubers. Included under *Fusarium sambucinum* Fckl. is a single specimen of *F. sambucinum* Fckl. f. 1 Wr.,* the other isolates of this fungus being the typical species.

* Kindly identified by Dr W. L. Gordon, Dominion Laboratory of Plant Pathology, University of Manitoba, Fort Garry, Manitoba.

Discussion

The results of this survey confirm the widely held view that *F. caeruleum* is the most important cause of dry rot in Great Britain. There is no doubt, however, that *F. avenaceum* is responsible for a small proportion of dry rot under natural conditions; indeed it is surprising that this fungus is not a more frequent cause of the disease owing to its evident pathogenicity and wide distribution in soil. This point is being investigated in a comparison of the parasitism of these Fusaria on potato.

Differences between corresponding figures for the 1949 and 1950 surveys and between these and the miscellaneous samples (Table 1) are not great, so that there is little evidence indicating a seasonal variation in the proportions in which the pathogens occur. Comparison of varieties shows that in King Edward a much higher proportion of the dry-rot lesions is caused by *F. avenaceum* than is the case in the other common varieties, which is in agreement with the observations of Moore (1945). This could indicate either that King Edward is specially susceptible to *F. avenaceum* or that it is somewhat resistant to *F. caeruleum*, or, of course, a combination of these effects. General observations on the incidence of dry rot under commercial conditions, over a period of years, have shown that King Edward is relatively resistant to the disease as a whole, and this suggests that resistance to attack by *F. caeruleum* is in fact more marked than resistance to *F. avenaceum*. In Home Guard, another variety somewhat resistant to dry rot, *F. caeruleum* was isolated from a smaller proportion of the lesions than usual, but this may have been due either to the high percentage of isolation failures or to the relatively small number of samples examined.

Comparatively few species of *Fusarium* were encountered in this survey of dry rot in seed potatoes, considering the number which have been recorded from abroad. Two *Fusarium* species other than those noted above have been isolated from ware potatoes, namely *F. solani* (Mart. pr.p.) App. & Wr., from a sample of tubers affected with pink rot, and *F. oxysporum* Schl., from a small lesion at the stem end of a Doon Star tuber. *F. solani* is usually considered to be incapable of causing a tuber rot (Pethybridge & Lafferty, 1917), although it has been reported (Goss, 1940) that certain strains of this fungus are pathogenic to both potato stems and tubers. *F. oxysporum* has been found causing a wilt of potato plants in Britain and has been shown to cause a tuber rot under laboratory conditions (Chona, 1932).

In view of the importance of *F. sambucinum* Fckl. f. 6 Wr. as the cause of storage losses in North America, it is interesting to note that it was not found in this survey, although *F. sambucinum* and *F. sambucinum* f. 1 were isolated. Wollenweber and Reinking (1935) describe *F. sambucinum* f. 6 as having a cosmopolitan distribution, but there is only one British record—on mushroom (Wood, 1937). It is recorded on the continent of Europe, often as *F. sulphureum* (e.g. Gram, 1945), but does not appear to be as serious a parasite there as it is in North America.

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DRY-ROT DISEASE OF THE POTATO

III. A BIOLOGICAL METHOD OF ASSESSING SOIL INFECTIVITY

By R. K. McKEE and A. E. W. BOYD*

Agricultural Research Council, Potato Storage Investigation, c/o University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leicestershire

(With 1 Text-figure)

Living tubers were inoculated by a standard method with small quantities of the soil to be tested and held under conditions suitable for infection. The number of dry-rot lesions that developed was regarded as giving a measure of the soil infectivity; this number, expressed as a percentage of the total number of inoculations, was termed the 'Infectivity Index' of the soil.

In these experiments, 91% of the lesions were caused either by *Fusarium caeruleum* or by a few other *Fusarium* species of which much the most common was *F. avenaceum*. In most instances it proved possible to distinguish the lesions caused by these two groups of fungi in tubers of the variety Doon Star by the colour of the rotted tissues as seen on cutting; isolations showed that 85% of the separations made in this way were correct.

Data are given indicating the sensitivity and consistency which may be expected from the method.

The incidence of dry rot in commercial stocks of seed potatoes varies greatly, not only in different varieties but also in different stocks of the same variety in any one season. In studying the reasons for this variation, we have investigated the infectivity of a number of field soils and its relation to the amount of dry rot occurring naturally in tubers of susceptible varieties grown in those soils. In the course of this work it became necessary to develop further the biological method of assessing soil infectivity used by Foister, Wilson & Boyd (1945); the technique, which has been found suitable for the examination of large numbers of samples, is described in this paper.

Methods

Material

The variety Doon Star was selected as suitable for inoculation on account of its susceptibility to dry rot and the regular shape of its tubers. The tubers were dipped, as soon as possible after lifting, in a 2% solution of formalin for 30 sec. to prevent natural infection through wounds caused during harvest. After drying, the tubers were stored in large paper sacks. Chance infection following dipping was rare (0·2% average over three seasons' work).

* Now Plant Pathologist, Edinburgh and East of Scotland College of Agriculture, George Square, Edinburgh.

Inoculations were made, as far as possible, in mid-winter, as it has been found that variations between and within samples are most liable to occur in freshly dug and in sprouting tubers.

Soil samples

Representative samples were obtained by taking a number of cores evenly distributed over the whole of the field under test. For fields up to 5 acres, fifty cores were taken to a depth of about 4 in.; this number was increased to 100 for fields over 5 and under 10 acres and so on. The soil corer was sterilized between fields but not between separate cores. The cores from any one field were bulked, well mixed and sieved and an aliquot portion taken.

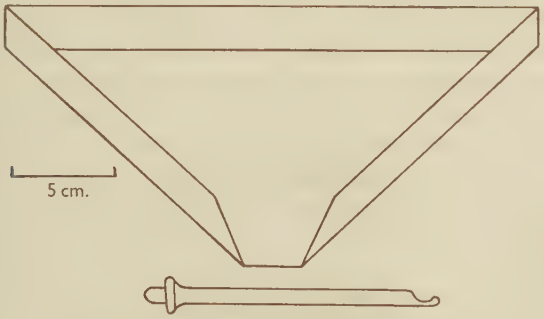

Fig. 1. Metal tray and glass inoculator.

Comparatively small quantities of soil were required for each test, and it was found convenient to store the samples in large boiling tubes, plugged with cotton waste or similar material. When the soil was required for inoculation it was poured into a small, triangular, metal tray (Fig. 1) which could be sterilized by dipping in alcohol and flaming. The tray was shaped so that any surplus soil remaining after inoculation could easily be returned to the boiling tube.

Inoculation

Inoculations were made with a glass inoculator (Fig. 1), the rounded end of which was pressed into the tuber until stopped by the flange; a spoonful of the air-dry soil under test was picked up with the other end, poured into the hole and pressed down so that all the soil was moistened with the sap from the damaged tuber cells. The use of the spoon ensured that approximately equal amounts of soil (0·1 g.) were taken for each sample. The inoculators were sterilized after each inoculation by immersion in alcohol.

To determine the degree of infectivity of a particular soil, 100 inoculations were made, two on each of fifty tubers. It was found that more than two infections per tuber made subsequent counting difficult owing to coalescence of the lesions. The fifty inoculated tubers were then placed in a small potato planting box,

which also held the unwounded and wounded uninoculated tubers included as a control to detect extraneous infection. It was found that chance infection was not serious, even when the tubers were stored in such open boxes; during the course of the work, out of 2476 uninoculated wounds twenty-eight became infected, seven only of these (0·3%) by *Fusarium caeruleum* (Lib.) Sacc.

The boxes of tubers were incubated at 60° F., humidity being kept as near saturation as possible for the first week by covering the boxes with wet sacks. As a check on the suitability of environmental conditions for infection, a few boxes of tubers inoculated with a suspension of spores of *F. caeruleum* were included with all series of soil inoculations.

Examination and classification of lesions

After 6 weeks' incubation at 60° F., lesions were sufficiently large to be detected easily when the tubers were cut across; a longer period than this might have given a slight increase in the number of lesions noted but it would also have allowed coalescence of lesions from opposite sides of the tubers and the development of secondary rots. The number of successful inoculations, expressed as a percentage of the total number of inoculations, was termed the 'Infectivity Index'.

Considerable numbers of soil-borne fungi have been recorded as causing rots of potatoes, and the possibility had to be considered that even more might produce lesions under the artificial conditions of inoculation employed. Isolations from inoculated tubers showed, however, that actively spreading lesions were caused, with few exceptions, either by *F. caeruleum* or by a few other *Fusarium* species of which much the most common was *F. avenaceum* (Fr.) Sacc. It was found to be impossible, without cultural study, to differentiate between the lesions caused by the species other than *F. caeruleum*; these, therefore, will be referred to collectively as the '*F. avenaceum* group'.

In examining the inoculated tubers it was necessary to distinguish the lesions caused by *F. caeruleum* from those caused by the '*F. avenaceum* group', if the index was to have any practical value, as the conditions of inoculation were apparently specially favourable for *F. avenaceum*—a fungus which is very widely distributed in cultivated soils but which, under natural conditions, causes only some 6% of all dry-rot infection (McKee, 1952). The Infectivity Index was, therefore, expressed specifically either as the *F. caeruleum* Index or as the '*F. avenaceum* group' Index. The latter term was often contracted to the *F. avenaceum* Index, as *F. avenaceum* was the predominant fungus in the group. The desirability of separating the two indices is demonstrated by the results given in Table 2.

In the variety Doon Star, the tuber tissue rotted by *F. caeruleum*, when freshly cut, has typically a pale fawn colour blending gradually at the margin into the colour of the healthy flesh. Darker bands are, however, often present in the older part of the lesions and the fawn colour darkens rapidly on exposure to the air. Surface sporodochia, which are often though not invariably produced, are white or blue if

formed in the dark and pale brick red if formed in the light. The blue mycelium, typical of *F. caeruleum*, is usually present at the base of these sporodochia and may also be found in cavities in the lesions. It should be noted that in other varieties the symptoms may be different—for instance, in the variety Arran Pilot the rotted tissue is much darker in colour.

Lesions in the variety Doon Star caused by the '*F. avenaceum* group' are dark chocolate brown in colour, often with a sharp margin between the healthy and diseased tissues; sporodochial formation is rare and only very occasionally is the typical red mycelium found in cavities.

It was found that the majority of the lesions in the variety Doon Star could readily be assigned either to the 'fawn' (*F. caeruleum*) or to the 'dark' class ('*F. avenaceum* group') but a few were mixed or intermediate in character and, visually, could be classed only as 'indefinite'. These might have been due either to a particular strain of the fungus or to a particular tuber giving an atypical reaction, to secondary infection with saprophytic organisms or to mixed infections—though the last-named have been found to be rare. Of the 4973 actively spreading lesions examined during this work, 71·5% were classed as 'fawn', 20% as 'dark' and 8·5 as 'indefinite'. Since the proportion of 'indefinite' lesions was usually small, it was decided to allocate them to the 'fawn' and 'dark' classes in the ratio in which these occurred in any particular soil.

A local necrosis, usually delimited by a well-defined callus layer, round the point of inoculation was often found in the absence of large actively spreading rots. These arrested lesions were associated with a number of saprophytic or weakly parasitic fungi. Thus *Rhizoctonia solani* Kühn was isolated from spherical cavities divided from the healthy tissues by a narrow cork layer. These cavities were usually filled with brown mycelium and cell detritus in which typical brown sclerotia were sometimes present. *Fusarium culmorum* (W. G. Sm.) Sacc. was often found associated with small dark lesions of irregular outline and well-defined margin (often with lens-shaped cavities); these were termed 'arrested dark lesions'. Every gradation between these and typical 'dark' lesions was found to occur. Data presented in Table 1 suggest that such lesions were in fact caused by fungi of the '*F. avenaceum* group' whose spread had been checked, and that *F. culmorum*, which is of doubtful pathogenicity (McKee, 1952), was probably a secondary invader.

The accuracy of the visual identification was checked by isolation from random samples of the classified lesions, either by plating out small pieces of rotted tissue from the margins of the lesions on potato dextrose agar or by examining the spores produced when fragments of the diseased tissue were kept in a moist atmosphere for a few days. Table 1 shows the results of such isolations made at intervals over a period of 3 years.

Of the actively spreading lesions from which isolations were made, 91% gave either *F. caeruleum* or one of the '*F. avenaceum* group'. These fungi may well have been present in even higher proportion, as failure to isolate any fungus was usually

due to severe contamination with bacteria assumed to be saprophytic; tubers showing bacterial rots were excluded. Moreover, *F. culmorum*, which, as stated above, does not cause an active rot but is often present as a saprophyte on rotted tissues, grows much faster on potato dextrose agar than either *F. caeruleum* or the '*F. avenaceum* group' and may thus have sometimes masked the presence of these fungi. In the many isolations made during this work, only in two instances did *F. caeruleum* and *F. avenaceum* grow out together from the same piece of tissue.

Table 1. *Fungi isolated from lesions visually classified*

Type of lesion	No. of lesions examined	*F. caeruleum* (%)	'*F. avenaceum* group' (%)	*F. culmorum* (%)	No growth and other fungi (%)
Fawn	228	84·5	8·8	3·1	4·8
Dark	213	5·6	87·0	5·2	2·3
Indefinite	63	30·2	49·2	7·9	12·7
Arrested dark	208	0·5	68·7	21·2	9·6

The species of the '*F. avenaceum* group' produced a cerise coloration on potato dextrose agar and were indistinguishable from one another until the agar cultures had been kept a considerable time, when typical spores were usually formed. The spores produced when small pieces of rotted tissue were kept in a moist atmosphere were not sufficiently characteristic to enable these species to be separated, though they could not be confused with the spores formed by *F. caeruleum* under the same conditions. *F. culmorum* was soon recognizable on potato dextrose agar, both by its faster growth and general appearance, but the first formed spores on tissue fragments were often atypical and could be confused with those of the '*F. avenaceum* group'.

An attempt was made to identify a number of isolates of the '*F. avenaceum* group' retained for further study. Of 118 such isolates, eighty-one were found to agree broadly with the description of *F. avenaceum*, ten with *F. arthrosporioides* Sherb., thirteen with *F. tricinctum* (Corda) Sacc., while fourteen could not be classified, usually owing to failure to produce spores. It is possible that other species were involved, as it is difficult to decide on the exact limits of *F. avenaceum* owing to its great variability. This difficulty is also experienced with *F. arthrosporioides* (which is considered to be possibly a variant of *F. avenaceum*), but *F. tricinctum* appears to be a distinct species. These three fungi are all capable of causing an active rot of tubers of the variety Doon Star under experimental conditions and have also been isolated from diseased tubers found in commercial stocks (McKee, 1952).

Consistency of results

The Infectivity Index cannot be taken as an exact figure but rather as a general indication of the level of infectivity of a particular soil. The consistency of results to be expected is shown in Table 2, in which are given five replicate estimations

(each based on 100 inoculations) of the Infectivity Index of each of nine field soils. In soils A–E, the five replicates were made in each case from a single sample of the soil in question, while in soils W–Z the replicates were made one from each of five entirely separate samples from each field.

TABLE 2. *Replicate estimations of the Infectivity Indices of nine field soils*

Replicate	A	B	C	D	E	W	X	Y	Z
				F. caeruleum Index					
1	43	1	33	20	18	1	5	41	5
2	29	1	43	23	10	1	8	50	4
3	36	2	41	36	27	1	6	43	5
4	30	2	40	29	13	0	8	50	7
5	26	2	27	16	10	0	10	39	1
Mean	32·8	1·6	36·8	24·8	15·6	0·6	7·4	44·6	4·2
				F. avenaceum Index					
1	2	16	6	1	4	2	3	3	0
2	0	14	3	1	7	10	2	2	1
3	1	21	3	0	7	3	10	5	1
4	2	24	1	3	5	2	6	6	3
5	1	6	4	0	3	4	3	2	4
Mean	1·2	16·2	3·4	1·0	5·2	4·2	4·8	2·6	1·8

TABLE 3. *Fiducial limits* ($P = 0{\cdot}05$) *of individual determinations* (*based on* 100 *inoculations*) *of the Infectivity Index* (F. caeruleum)

Value of *F. caeruleum* Index	Fiducial limits
0	0–2
2	0–7
7	2–15
15	7–26
26	15–38
38	26–52
52	38–65
65	52–77
77	65–87
87	77–95
95	87–99
99	95–100

Considerable variation was found between replicate estimations of the Infectivity Index of the same soil, but it is of interest to note that the variation between separate samples from the same field (soils W–Z) was no greater than that found in replicates of the same soil sample (soils A–E). It is possible that with further experience, more consistent results might be obtained by improving the laboratory method without increasing the number of inoculations above 100 per soil. The most important factor in obtaining reproducible results appears to be the maintenance of adequate humidity immediately after inoculation. An effect thought to be due to humidity variation is seen in Table 2. The no. 5 replicates were made at a time when the storage chamber was nearly empty, making humidity difficult to control,

whereas nos. 1–4 were made when the room was almost filled with boxes. It will be noted that the no. 5 Infectivity Index values are, in general, lower than the average.

The fiducial limits for individual determinations of the *F. caeruleum* Index have been calculated from the data given in Table 2; selected values are given in Table 3.

Sensitivity of isolation technique

The Infectivity Index may be used for comparing the infectivity of different soils but gives no information on the actual numbers of spores (or other infective particles) in a particular soil. Attempts to isolate *F. caeruleum* by dilution and plating on Waksman's Acid Agar proved unsuccessful, even though soil of a high infectivity was used. Indirect evidence as to the numbers of *F. caeruleum* spores necessary for various levels of infection, when inoculation was carried out under the same conditions as in examination of soils, was obtained in the following way. Dilution series of spore suspensions in sterile water were prepared and, by means of a dropping tube, single drops of known size (0·04 ml.) were placed in wounds made with the glass inoculator in tubers of the variety Doon Star. Care was taken to avoid sedimentation of the spores in the dropping tube. The counts of viable spores in the suspensions were obtained by plating 1 ml. portions of a suitable dilution on potato dextrose agar. Results of two experiments made on different dates and with different isolates of *F. caeruleum* are given in Table 4.

Table 4. *Infection resulting from the inoculation of potato tubers (var. Doon Star) with drops of spore suspensions containing known numbers of* F. caeruleum *spores*

Series A		Series B	
Viable spores per drop*	Number of lesions resulting from 100 inoculations	Viable spores per drop*	Number of lesions resulting from 100 inoculations
2300	98	242	73
460	97	121	74
92	94	60	75
18·4	72	30	74
3·7	11	15	69
0·74	8	7·5	55
0·15	6	3·75	51
0·03	1	1·87	34
0·006	0	0·94	19
Control	0	0·47	12
		Control	0

* Standard error: series A, ±6%, series B, ±4%.

It is evident that very small numbers of spores can cause infection and also that the proportion of successful inoculations depends, within each series, on the number of spores present, provided this is less than is necessary to obtain maximum infection. This problem of saturation infection has not arisen in the study of field soils, where the Infectivity Index values are usually low; it is suggested that the upper range might be extended by dilution with sterile sand.

Introduction of small quantities of unsterilized soil known to be free from

F. caeruleum, or of sterilized soil or sand with the drop of spore suspension, was not found to influence the result. However, only one sample of soil was tested and it is possible that other soils would be found to contain organisms antagonistic to *F. caeruleum* or to possess physical or chemical properties restricting the growth of this fungus.

An indication of a decrease in tuber susceptibility with age may be seen in the experiment quoted in Table 4. The A series of inoculations, made on dormant tubers (early March), showed a maximum of 98% infection, whereas the B series, for which sprouting tubers were used (late May), gave a maximum infection of 75% only. The result is of interest as susceptibility is usually considered to increase throughout the storage period.

Effect of storage of soil samples on the infectivity index

A considerable interval often elapsed between collection and test of a soil sample; during this period the soil was kept in an air-dry state, as it had been found that *F. caeruleum* remained viable for long periods under such conditions. Seven soils collected in autumn 1943 were stored air dry in closed containers and the *F. caeruleum* indices measured at intervals over 5 years. The results are given in Table 5. The general increase in the values of the Infectivity Indices over the period 1944–6 is probably an indication of improvements in method rather than actual changes in the soils: after 1946 survival decreased.

Table 5. *Effect of storage over a period of years on the* F. caeruleum *Indices of seven soils kept in an air-dry condition*

Soil no.	1944*	1946	1947	1948
11	88	100	70	60
6	52	72·5	46	5
9	32	60	50	34
1	16	40	2	1
18	4	35	16	10
5	0	7·5	4	0
16	0	0	2	0

* The figures for 1944 include the *F. avenaceum* Index.

It is interesting to contrast the viability in air-dry soil of *F. caeruleum* and *F. avenaceum*. As shown in Table 6, the latter loses its viability over a period of 1 year;

Table 6. *Comparison of the effects of storage, over 1 year, on the* F. caeruleum *and* F. avenaceum *Indices of six soils kept in an air-dry condition*

	F. caeruleum Index		*F. avenaceum* Index	
Soil	1947	1948	1947	1948
1C7	42	41	0	0
1C15	62	45	0	0
3C14	29	60	42	0
1B9	13	22	17	0
1B12	0	0	29	0
1B4	0	2	46	1

this behaviour may possibly be due to the fact that chlamydospores, produced in large numbers by *F. caeruleum*, are very rarely found in *F. avenaceum*, the predominant fungus in the '*F. avenaceum* group'.

The possibility of changes in the Infectivity Index during the period between collection and test of a sample (usually from 3 to 4 months) has not yet been investigated.

Discussion

The isolation of plant pathogenic fungi from the soil by plating methods is hindered in many cases by their slow growth in culture and by the presence of much larger numbers of fast-growing saprophytes. Pathogens may often be detected by exposing a susceptible host plant to the infested soil under conditions suitable for infection. A quantitative estimation of what may be called 'soil infectivity', is given, within limits, by the incidence of disease when large numbers of host plants are used. A number of instances in which this technique has been employed are quoted by Garrett (1944), while more recently, Wilhelm (1950), working with *Verticillium alboatrum*, has determined the 'Infection Index' of field soils using tomato seedlings as the test plants.

Storage organs are especially suitable for the isolation of fungi attacking them, as they can be kept under a wide range of environmental conditions and are easily handled. Pethybridge & Bowers (1908), Small (1944) and Lansade (1949) used wounded potato tubers as a selective substrate to demonstrate contamination of soil, tubers, stores, sacks, etc., by *F. caeruleum*; Lansade obtained a quantitative measure of the degree of contamination by counting the number of lesions developing on cut tubers after rubbing on the surface to be tested. Gibbs (1938) determined the presence of *Phoma lingam* by dipping wounded swede roots in aqueous suspensions of infective soil. Yarwood (1946) has shown that living carrot disks may be used for the isolation of *Thielaviopsis basicola* from the soil, although *Sclerotinia sclerotiorum*, to which carrots are very susceptible, could not be detected in this way with any regularity. *Phytophthora cinnamomi* has been isolated by Campbell (1949*a*) by inserting soil from the root area of short leaf pine (*Pinus echinata*) affected by little leaf, into holes bored in apples; the identity of the fungus was confirmed by plating pieces of the rotted apple tissue on maize meal agar. This method was also used to demonstrate that *Phytophthora cinnamomi* was much more abundant in the soil near trees affected with little leaf than in the soil of the root zone of healthy trees (Campbell, 1949*b*).

Data given in this paper show that potato tubers may be used to detect the presence of very small numbers of *Fusarium caeruleum* spores; inoculation with about fifteen spores (in sterile water) gave 70% infection, a figure much higher than the *F. caeruleum* Index of most field soils. In determining the Infectivity Index, about 0·1 g. of soil was inserted for each inoculation; 150 'spores' per gram could thus be regarded as rendering a soil highly infective provided antagonism by other soil micro-organisms was no more severe than that found where unsterilized soil was added with the spore suspension. In direct isolation of fungi from the soil, by

plating, a minimum dilution of 1:2000 is usually necessary; at this dilution a colony of *F. caeruleum*, if present to the extent mentioned, could be expected no more than once in every thirteen plates.

It has been shown that the chance of success in an inoculation is dependent, within limits, on the number of spores or other fungal particles present, so that the method of assessing infectivity is capable of giving a rough quantitative estimation of the infestation of a soil, provided the influence of other factors such as virulence of the pathogen and biological characteristics of the particular soil are discounted. The data obtained so far, however, are inadequate to form any definite conclusion on the importance of these factors, and until further work is done the Index must be regarded as a measure of infectivity rather than infestation.

It would appear that a heavy infestation by the '*F. avenaceum* group' may sometimes mask the presence of *F. caeruleum*; thus in Table 6, fields 3C14 and 1B9 showed a rise in the *F. caeruleum* Index after storage of the air-dry soil for a year, during which time the originally large *F. avenaceum* population had died out. This effect, which is regarded as due to competition rather than to antagonism between the fungi, will have little influence on the *F. caeruleum* Index in most soils, but could be allowed for where *F. avenaceum* is prevalent.

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A DISEASE OF *SCABIOSA CAUCASICA* CAUSED BY THE NEMATODE *APHELENCHOIDES BLASTOPHTHORUS* N.SP.

By MARY T. FRANKLIN

Rothamsted Experimental Station, Harpenden, Herts

(With Plate 2 and 2 Text-figures)

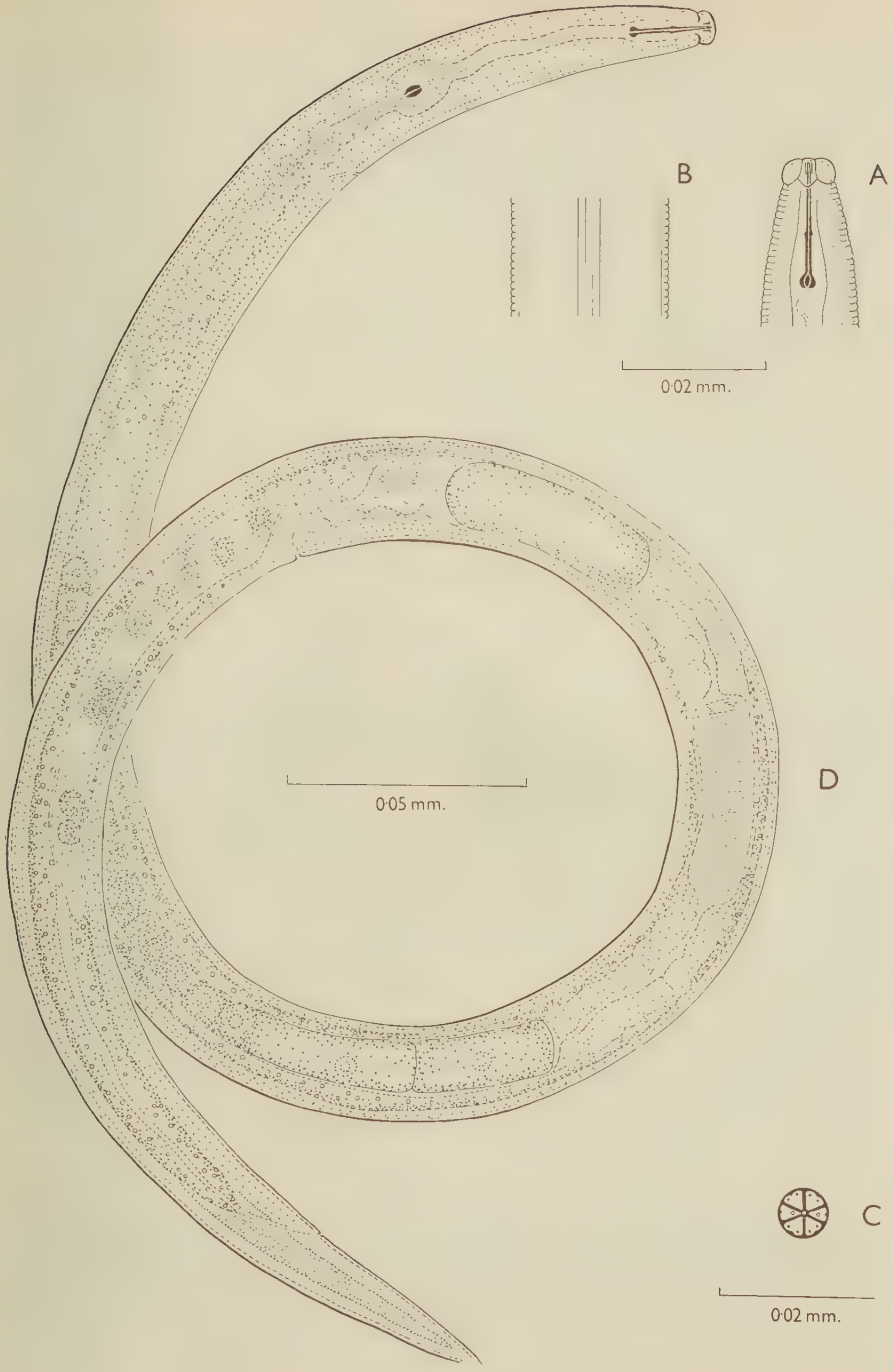
Scabiosa caucasica has been found affected by a new species of *Aphelenchoides*, herein described and named *A. blastophthorus*. The symptoms of disease are destruction of the inflorescences and distortion of the leaves with reduction of the laminae. Similar symptoms were produced in scabious by experimental infestation. The nematode can infest teazle (*Dipsacus fullonum*), but has not been found naturally occurring on any host but scabious.

A disease of *Scabiosa caucasica*, variety Clive Greaves, being grown commercially at Leicester, was first observed by the grower in 1946, and late in the summer of 1949 it was brought to the notice of Mr J. H. White of the N.A.A.S., Shardlow, Derby. The disease is characterized by the death of the young inflorescences with consequent 'blindness' of the plants. A few nematodes resembling *Aphelenchoides parietinus* (Bastian, 1865) Steiner, 1932, were found close to the dead buds, but their numbers did not seem to be sufficient to be the cause of the disease, nor is this species generally regarded as a plant parasite. In 1950 the disease reappeared at the same nursery. In July numbers of nematodes were found in the buds, and specimens were sent for identification to the Nematology Department, Rothamsted Experimental Station.

The nematodes are closely similar to *A. parietinus*, but do not altogether fit Bastian's original description of this species (Bastian, 1865). They are considered to belong to a hitherto undescribed species which is here named *A. blastophthorus* from the Greek *βλαστός*, a bud and *φθόρος*, destroying.

Aphelenchoides blastophthorus n.sp.

The cuticle has fine annulations, slightly less than 1 μ wide. The lateral field is marked by four incisures and is about one-seventh of the body width across (Text-fig. 1 B). In both sexes the tail tapers gradually and ends in a simple mucron. The anterior end of the body narrows gradually to rather less than half the greatest body width, and there is a constriction at the base of the lips. A head-on view shows six lips fused together, with indications of two papillae on each of the two dorso-laterals and two ventro-laterals and a faint suggestion of amphidial openings on the lateral lips (Text-fig. 1 C). A lateral surface view of the head is characteristic, showing clearly the posterior margins of the lips, which are obtusely pointed



Text-fig. 1. *Aphelenchoides blastophthorus* n.sp. A, head showing stylet and lip region; B, lateral field; C, diagram of head-on-view; D, whole female.

(Text-fig. 1 A). The mouth stylet, which averages 17 μ long, has three distinct basal knobs. The oesophagus is typically aphelenchoid, with well-defined valvate median bulb and elongated dorsal gland. The intestine ends in a narrow rectum a little longer than the greatest body width. The nerve ring encircles the posterior part of the oesophagus at about one bulb-length behind the oesophageal bulb, and the excretory duct opens ventrally at about the level of the anterior edge of the nerve ring.

Female. Length 830 μ (680–900 μ, *n* 39), breadth 22 μ (16·7–26 μ, *n* 35), $a = 38$ (32–47, *n* 30), $b = 10{\cdot}2$ (9·3–11, *n* 9), $c = 19$ (16–21, *n* 9), V = 70% (68–74%, *n* 14).

The anterior end of the ovary lies close behind the end of the oesophageal gland, the oocytes being arranged in a single series. Sperms are often seen closely packed in the uterus and in the post-vulval sac, which extends to about half the distance from vulva to anus. Not more than one egg at a time has been seen in the uterus. The tails of nine specimens varied from 42 to 48·6 μ in length, averaging 45 μ.

Male. Length 820 μ (670–910 μ, *n* 39), breadth 19 μ (16·7–23·3 μ, *n* 35), $a = 41$ (35–47, *n* 30), $b = 9{\cdot}5$ (7·2–10·7, *n* 9), $c = 16$ (14–19, *n* 9). Spicules, dorsal limb 28 μ (24–31 μ, *n* 13), ventral limb 16 μ (14–19 μ, *n* 13). Stylet 17·1 μ (15–19·5 μ, *n* 27, males and females together).

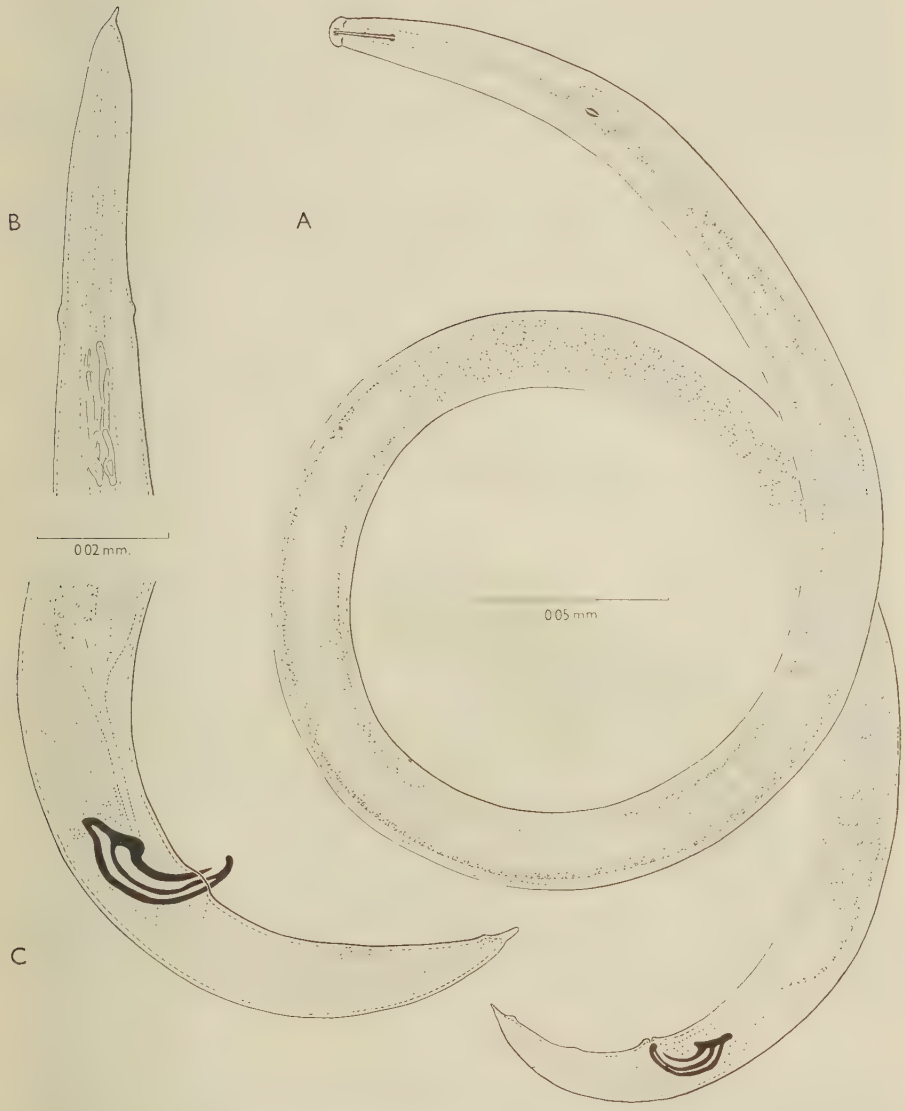
The testis reaches to the posterior end of the oesophageal gland; the cells appear to be in double series and the vas deferens joins the rectum to form a short cloaca. The spicules are rather large and typically aphelenchoid; the arc formed by the dorsal limb has a characteristic flattening about midway (Text-fig. 2 C). The tip of the ventral limb is sharply pointed, but that of the dorsal and stouter limb has a slight knob or hook. The tails of nine worms varied from 45 to 59 μ long, averaging 52 μ. There are three pairs of papillae, one pair at the base of the terminal mucron, one midway along the tail and one situated ad-annally (Text-fig. 2 B, C). On being killed by gentle heat the tail takes on the shape of an open hook of about the same curvature as that of *A. fragariae*.

Eggs and larvae. The eggs are approximately 60 × 22 μ and are laid in an unsegmented condition. Larvae have not been studied in detail but show no striking characteristics.

Movement. In water the nematodes move in the typically aphelenchoid way by which they are able to progress by means of sinuous, slightly spiral, waving movements. A comparison between *A. blastophthorus* and *A. ritzema-bosi* in water showed that the former moves distinctly more slowly than the latter.

Relationships

The nematode parasitic on scabious differs from all other known species of *Aphelenchoides* and must be considered a new species. It differs from *A. fragariae* in being distinctly larger and in having larger spicules and mouth stylet. From *A. ritzema-bosi* and *A. ribes* it differs in: (1) being slightly shorter, though of the same breadth relative to its length; (2) the more anterior position of the excretory



Text-fig. 2. Male *Aphelenchoides blastophthorus* n.sp. A, whole nematode; B, tail seen ventrally showing papillae and spicules; C, lateral view of tail.

pore, which in both the other species is at least three bulb-lengths behind the centre of the oesophageal bulb as compared with less than two in the new species; (3) the much less pronounced curvature of the male tail on killing by heat; (4) the simple mucron on the tail as compared with the irregular and often three- or four-pointed, or jagged mucron in *A. ritzema-bosi* and *A. ribes*; (5) the larger spicules which are less sharply bent and have a slight hook or knob on the tip of the dorsal limb.

From *A. parietinus*, as described by Bastian, *A. blastophthorus* is distinguished by its greater length and more slender form ($a = 38$ as compared with 23 in *A. parietinus*), and by the heavier and distinctly knobbed stylet. The names of thirteen other species of *Aphelenchoides* and *Aphelenchus* are given by Goodey (1951) as synonyms of *Aphelenchoides parietinus*; some are described as plant parasites, but all differ from the present species either in the size and shape of the stylet, or in the length or relative breadth of the worm. In many cases the original descriptions are inadequate and it seems possible that a careful study of *A. parietinus*, as it is now known, may show that it comprises more than one distinct species. However, none of the species which have been synonymized with *A. parietinus* can be considered identical with *A. blastophthorus*.

Bionomics

In June and July *A. blastophthorus* can be found in diseased scabious in considerable numbers in the axils of the leaves, around damaged leaf buds and amongst and within floret buds. It is then not uncommon to find from twenty to fifty of the nematodes of all ages in a leaf axil. They can be found in smaller numbers in this situation throughout the summer and until the plants die down in winter. In stained specimens of different parts of an infested plant taken in July approximately equal numbers of eelworms were found to be ectoparasitic and endoparasitic, most of the latter being in the tissues surrounding the lower part of the midrib of the crown leaves and only very occasional individuals being in the lamina, even of markedly distorted leaves. They have also been found occasionally within the stems of aborted inflorescences. In January and February numbers of the nematodes were found in the dead leaf bases and a few within the small living leaves in the centre of the crown. In severely affected inflorescences many or all of the florets fail to develop and nematodes of all ages may be found in them.

Effect on host

The symptoms of attack on scabious are most noticeable in July when the plants should be flowering freely. Infested plants then bear damaged, distorted or aborted inflorescences which may be represented by nothing more than a small mass of blackened tissue between the pair of leaves terminating the inflorescence stem. In the worst cases the plants may be completely blind. The less severely affected inflorescences often develop unevenly and the scales normally present

between the florets may be reduced in size and number. The leaves are distorted, twisted and puckered, with thickened midribs and often severely reduced laminae which are somewhat thicker and tougher than normal (Pl. 2, fig. 1). Diseased plants are sometimes slightly darker green than healthy; this has been noticed particularly where growth of the plants is most luxuriant. Sometimes a narrow, brown, roughened band may be seen on the stem above the axil, suggesting damage by the feeding of the nematodes in the leaf axil before the elongation of that part of the stem and recalling the 'feeding areas' often seen on the leaves of strawberry plants infested with nematodes. The symptoms of attack often tend to disappear later in the season when the plants produce normal flowers and grow away from the disease.

Experimental infestations

Two 1-year-old scabious plants from a clean stock were potted and one was inoculated on 18 August and 14 September 1950 with a suspension in water of several hundreds of eelworms from a diseased scabious. By November both plants had produced inflorescences which failed to mature because of the lateness of the season. Those on the uninoculated plant appeared quite healthy and one or two of the outer florets expanded, but on the inoculated plant the inflorescences were brown and dead and were found to contain many *A. blastophthorus*. In the following spring the inoculated plant showed some distortion of the leaves and one of the inflorescences was much reduced and completely failed to develop (Pl. 2, fig. 2).

The new *Aphelenchoides* has not been found occurring naturally on any other host, although many weeds growing amongst diseased scabious have been examined, but an attempt was made to infest teazle (*Dipsacus fullonum*) since this is a nearly related plant. A young seedling teazle in a pot was inoculated on 12 September 1950 with a suspension in water of fifty nematodes from scabious and, on 13 September 1950, 100 more worms were added. This plant was kept in a warm greenhouse during the winter and slight distortion of the new leaves was noticed. At the beginning of May 1951, a critical examination of the plant showed that in some leaves the laminae were bent back from the midrib and the affected leaves were rather tougher than those of uninoculated plants. The aerial parts were torn apart and placed in a Baermann funnel on 5 May, when many *Aphelenchoides blastophthorus* of all ages were recovered. Slight brown lesions were observed on the stem of the plant for a short distance above some of the leaf axils, the buds of which were found to contain many eelworms of all ages, including eggs. Portions of the plant were stained in cotton blue-lactophenol, but no eelworms were found internally. From a consideration of the symptoms associated throughout the year with the presence of *A. blastophthorus* of all ages in scabious and in teazle, and of the results of the inoculation experiments, it is obvious that this nematode can be a parasite of both plants.

Occurrence

The type locality where the nematodes here described and named were found is a flower nursery at Leicester. Damage of a similar kind to caucasian scabious grown commercially, and associated with the presence of this *Aphelenchoides* species, has been reported also from Iver, Bucks; Crowland, Lincs; Wolverhampton, Staffs; Wisbech, Cambs; and Fareham, Hants. In July 1936, damage was observed to scabious at Combe-in-Teignhead, Devon, associated with an eelworm identified as *A. parietinus* (Anon, 1937). The losses were considerable and damage in this case was described as deformation of the flowers, which were green. It was not decided whether this condition was due to the nematodes or to some other cause. Having regard to the similarity between *A. blastophthorus* and *A. parietinus* it seems possible that the nematodes may have been of the new species and also the cause of the trouble, though the symptoms were not identical with those recently observed.

It is obvious that the standard commercial practice of propagating scabious by division of the plants could be a means of spreading the parasite.

Thanks are due to Mr J. H. White for the introduction to the nursery where the disease was first observed, and to the grower for facilities readily given; also to other N.A.A.S. officers for sending infested material. The photographs were taken by Mr J. H. White (Pl. 2, fig. 1) and Mr C. C. Doncaster (Pl. 2, fig. 2) of the Nematology Department, Rothamsted Experimental Station, to whom thanks are tendered.

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EXPLANATION OF PLATE 2

Fig. 1. Diseased scabious plants showing distorted leaves and poorly developed or aborted inflorescences.

Fig. 2. Inflorescences from left, control plant, right, plant inoculated with *Aphelenchoides blastophthorus* n.sp. on 18 August and 14 September 1950. Photo., 19 June 1951.

(*Received* 4 *August* 1951)

Fig. 1.

Fig. 2.

STUDIES IN *RUBUS* VIRUS DISEASES

II. THREE TYPES OF VEIN CHLOROSIS OF RASPBERRIES

By C. H. CADMAN*

Scottish Raspberry Investigation (East Malling Research Station), University College, Dundee

(With Plates 3 and 4)

Three diseases characterized by vein chlorosis of varying grades of severity are shown to be graft transmissible to a wide range of raspberry varieties. The diseases are thought to be caused by related strains of a virus transmissible by *Doralis* (*Aphis*) *idaei* V. d. G. and rarely, if at all, by *Amphorophora rubi* Kalt. It is proposed to refer to the diseases and viruses respectively as mild, moderate and severe vein chlorosis.

In an earlier review (Cadman & Harris, 1951), raspberry viruses were grouped under two categories. Those of group I induce visible mosaic symptoms of some description on a wide range of varieties of *Rubus idaeus* L., whilst those of group II do not. This grouping is empirical and does not imply any other character common to the viruses of each group.

The three diseases described in the present paper commonly occur in commercial stocks of raspberries and are caused by group I viruses. The symptoms of all three are similar in type and most aptly described by the names mild, moderate and severe vein chlorosis. There is some evidence that the diseases are caused by related strains of one virus. Cadman & Hill (1947) showed that *Doralis* (*Aphis*) *idaei* V. d. G. was a vector of moderate vein chlorosis, but the relations of this aphid to the mild and severe diseases have not yet been established.

The techniques used throughout the present work were described earlier (Cadman, 1951).

Symptoms on Norfolk Giant

Mild vein chlorosis is characterized by faint yellowing of the tissue bordering the smaller leaf veins, occasionally coalescing into small diffuse patches (Pl. 3, fig. 1). Symptoms are often localized and generally difficult to detect on this variety. They are not masked in warm weather.

Moderate vein chlorosis symptoms are similar to those just described. They differ in being more generally distributed and much more conspicuous (Pl. 3, fig. 2), but are also not masked in warm weather.

Severe vein chlorosis is characterized by intense chlorosis of the smaller leaf

* Now at Scottish Horticultural Research Institute, Mylnefield, Invergowrie, by Dundee.

veins and adjacent tissue, regularly coalescing into large and conspicuous patches (Pl. 3, fig. 3). Severely affected leaflets are frequently distorted and curled. Symptoms are systemically distributed and are not masked in warm weather.

TRANSMISSION

Mild vein chlorosis

Mild vein chlorosis has not been identified on commercial stocks of Norfolk Giant in Scotland but developed on plants of this variety grafted, in 1948 and 1949, to 'healthy' plants of Baumforth's Seedling B. All 'healthy' plants of Baumforth's B handled since 1944 showed mild vein chlorosis symptoms (Pl. 3, fig. 4), and these appear also in the photographs of Harris (1933) illustrating his type *b* symptoms. Though they were for long assumed to be a normal varietal characteristic, the results of the experiments shown in Table 1 demonstrate that the vein chlorosis symptoms on Baumforth's B are of virus origin. Symptoms on Norfolk Giant were extremely mild and difficult to distinguish. Prentice & Harris (1950) also reported the appearance of slight vein chlorosis on plants of Norfolk Giant grafted to Baumforth's B, and attributed this to an unsuspected virus in the latter. Symptoms on plants of Lloyd George (New Zealand stock) and St Walfried grafted to Baumforth's B in 1946 and 1948 were more conspicuous and were, in fact, indistinguishable from those of moderate vein chlorosis on these varieties.

TABLE 1. *Results of graft transmissions of mild vein chlorosis virus from Baumforth's B infectors*

Indicator	No. of plants	Year of graft	Mild vein chlorosis	No symptoms
St Walfried	12	1946	11	1
St Walfried	5	1948	5	0
Norfolk Giant	10	1948	6	4
Norfolk Giant	4	1949	4	0
Lloyd George	3	1948	2	1

Mild vein chlorosis symptoms appeared in 1945 on two of four healthy Norfolk Giant plants to which large numbers of *D. idaei* had been transferred after feeding for 3 weeks on four Baumforth's B plants infected with mosaic 2 disease. No such symptoms developed on four Norfolk Giant plants that received *Amphorophora rubi* fed on similar infectors or on four control plants that did not receive aphids. This result indicates that *Doralis idaei* may be a vector of mild vein chlorosis, but it has not been confirmed.

Moderate vein chlorosis

Moderate vein chlorosis commonly occurs in commercial stocks of Norfolk Giant and was shown to be graft-transmissible in 1944. Moderate vein chlorosis developed on nine out of ten healthy plants of Norfolk Giant after grafting to infected canes of this variety, whereas ten similar but ungrafted controls remained healthy. Other

experiments (Table 2) showed that this disease was transmissible to, and produced vein chlorosis symptoms on, Lloyd George, St Walfried, Burnetholm Seedling and the Malling varieties Enterprise, Jewel, Notable, Promise, Exploit and Seedlings M and Z (Pl. 4, fig. 5). Some of these transmissions were subsequently checked by back grafts to Norfolk Giant. Plants showing vein chlorosis selected from commercial stocks of Lloyd George, St Walfried, Malling Promise, and a rogue variety, at one time common among commercial stocks of Norfolk Giant, all produced moderate vein chlorosis symptoms on the healthy Norfolk Giant indicators to which they were grafted.

TABLE 2. *Results of graft transmissions of moderate vein chlorosis*

Indicator	No. of plants	Infector	Year of graft	Moderate vein chlorosis	Mosaic 2	No symptoms	Moderate vein chlorosis in check tests
Norfolk Giant	10	Norfolk Giant	1944	9	—	1	—
Norfolk Giant	5	Norfolk Giant	1949	5	—	0	—
Norfolk Giant	10	Controls	1944	0	—	10	—
Norfolk Giant	20	Lloyd George	1945	14	3	3	—
Norfolk Giant	4	Lloyd George	1946	1	3	0	—
Norfolk Giant	4	St Walfried	1946	2	—	2	—
Norfolk Giant	5	Malling Promise	1946	1	2	2	—
Norfolk Giant	6	Malling Landmark	1946	6	—	0	—
Lloyd George	5	Norfolk Giant	1948	5	—	0	—
St Walfried	5	Norfolk Giant	1948	5	—	0	—
Burnetholm Seedling	4	Norfolk Giant	1947, 48	3	—	1	1/4
Malling Enterprise	3	Norfolk Giant	1948	3	—	0	—
Malling Jewel	3	Norfolk Giant	1948	3	—	0	—
Malling Seedling K	3	Norfolk Giant	1950	2	—	1	—
Malling Landmark	10	Norfolk Giant	1947	10	—	0	5/6
Malling Landmark	3	Norfolk Giant	1948	3	—	0	1/1
Malling Landmark	3	Controls	1947	0	—	3	0/3
Malling Seedling M	3	Norfolk Giant	1948	3	—	0	—
Malling Notable	9	Norfolk Giant	1948	7	—	2	—
Malling Promise	10	Norfolk Giant	1946, 48	6	—	4	6/7
Malling Exploit	5	Norfolk Giant	1949	5	—	0	—
Malling Seedling Z	5	Norfolk Giant	1949	5	—	0	—
Newburgh	3	Norfolk Giant	1948	0	—	3	0/2
Cuthbert	7	Norfolk Giant	1948, 49	0	—	7	0/7
Latham	5	Norfolk Giant	1949	0	—	5	0/5
Viking	5	Norfolk Giant	1949	0	—	5	0/5

Anomalous results were obtained with Malling Landmark, Malling Seedling K, varieties of North American origin and Baumforth's Seedling B and will be considered in that order.

Plants of Malling Landmark and Seedling K grafted to infected Norfolk Giant developed complex mosaic symptoms in which severe chlorotic blotching, due to the leaf mottle virus (Cadman, 1951) present in the infectors, predominated (Pl. 4, fig. 6). Transmission of the vein chlorosis disease to the Malling Landmark plants was confirmed by back grafts to Norfolk Giant.

Despite successful graft unions between infected Norfolk Giant scions and plants of the North American varieties Newburgh, Cuthbert and Viking, these varieties failed to show vein chlorosis symptoms and no evidence of transmission was obtained. These results are at present uninterpretable.

TABLE 3. *Results of grafts of Norfolk Giant and Baumforth's B to moderate vein chlorosis infectors*

		Indicator					
		Norfolk Giant			Baumforth's B		
Infector	Year of graft	Symptom, vein chlorosis	Symptom, mosaic 2	No symptoms	Symptom, curly dwarf	Symptom, mosaic 2	No symptoms
Malling Landmark	1946	6	—	—	—	—	5
Norfolk Giant	1944–9	14	—	1	15	—	3
Norfolk Giant rogue	1945	2	1	1	4	3	—
Lloyd George	1945	14	3	3	1	4	—

The results of experiments with Baumforth's B are given in Table 3. Three samples of infected Malling Landmark plants from commercial stocks were grafted to healthy plants of Norfolk Giant and Baumforth's B in 1946. Although all the former developed moderate vein chlorosis, no symptoms additional to mild vein chlorosis developed on the Baumforth's B plants, although at least three of the graft unions were sound. On the other hand, numerous grafts of infected plants of Norfolk Giant, Norfolk Giant rogue and Lloyd George to Baumforth's B resulted mainly in the development of curly dwarf* disease (Prentice & Harris, 1950). The sample of Norfolk Giant listed in Table 3 includes the ten infectors referred to in Table 1, additional plants of the same origin and others infected from these by grafting. Mosaic 2 disease was present in several of the Norfolk Giant rogue and Lloyd George plants. These results are discussed on p. 67.

Cadman & Hill (1947), in experiments with *Doralis idaei* described in greater detail below, reported the transmission of a vein chlorosis disease from Lloyd George to Norfolk Giant. The symptoms were those of moderate vein chlorosis but were then ascribed to the curly dwarf virus of Prentice & Harris (1950). In a preliminary experiment in 1945, moderate vein chlorosis symptoms developed on three of six healthy Norfolk Giant plants that received *D. idaei* fed for 17–20 days on Lloyd George infectors. No such symptoms were observed on six control plants that did not receive aphids or on three plants that received *Amphorophora rubi* previously fed for 18 days on similar Lloyd George infectors.

The results of the 1946 experiment, published in the note referred to above, are shown again in Table 4. Large numbers (*c.* 1000 or more) of *Doralis idaei* and *Amphorophora rubi* were allowed to feed on infected Lloyd George plants for the periods stated before being transferred to sets of ten plants each of St Walfried and

* This disease bears no relation to the 'curly dwarf' of Rankin (1927).

Norfolk Giant. Moderate vein chlorosis symptoms subsequently developed on some of the plants that received *Doralis idaei* and on a single Norfolk Giant plant that received *Amphorophora rubi* fed for 7 days on the infectors.

TABLE 4. *Transmission of moderate vein chlorosis from Lloyd George by aphids*

No. aphids per plant: ∞. Test feeding period: 3 and 6 days (bulked).

		I.F.P.				
Aphid sp.	Indicator	12 hr.	3 days	7 days	14 days	21 days
D. idaei	Norfolk Giant	0/10	1/10	1/10	2/10	2/10
	St Walfried	2/10	0/10	3/10	3/10	4/10
A. rubi	Norfolk Giant	0/10	0/10	1/10	0/10	0/10
	St Walfried	0/10	0/10	0/10	0/10	0/10

No vein chlorosis symptoms developed on the remaining plants of the *A. rubi* series, or on twenty control plants of Norfolk Giant and St Walfried that received aphids from the stock cultures. As the symptoms observed on the Norfolk Giant and St Walfried plants in these experiments were indistinguishable from those of the graft-transmissible disease described above, the causal viruses were presumed to be identical.

Severe vein chlorosis

Severe vein chlorosis was first found on plants of Norfolk Giant in eastern Scotland in 1944 and later, in 1945, transmitted to healthy plants of this variety by grafting. Symptoms on the latter qualitatively resembled those of mild and moderate vein chlorosis but were consistently more severe and conspicuous (Pl. 3, fig. 3). Both the infector and indicator plants of this experiment were subsequently found also to contain leaf mottle and mild yellows (Cadman & Harris, 1951) viruses. Combinations of leaf mottle, mild yellows and moderate vein chlorosis or of the first two of these with veinbanding (Cadman & Harris, 1951) did not, however, induce severe vein chlorosis symptoms on Norfolk Giant. It was therefore concluded that the latter were caused by a virus distinct from any of these. As the plants from the 1945 experiment were used as infectors in subsequent grafting experiments, the presence of contaminant viruses complicates the interpretation of results.

Ten plants of Lloyd George (New Zealand stock) grafted, in 1947 and 1948, with infected Norfolk Giant scions developed a severe chlorotic disease the year after grafting. Growth of young canes was stunted and the leaves were markedly curled and distorted by conspicuous patches of chlorosis (Pl. 4, fig. 7). Severe vein chlorosis was detected in one of these plants by test grafts to Norfolk Giant. Whilst the leaf symptoms on the Lloyd George plants were probably due to the mild yellows and severe vein chlorosis viruses, it is uncertain whether the effects on cane growth can be attributed solely to these viruses. Similar, though more severe, symptoms developed on eight plants of a commercial stock of Lloyd George, known

to be infected at least with severe mosaic 2 and mild yellows, grafted with infected Norfolk Giant (Table 5).

TABLE 5. *Results of graft transmissions of severe vein chlorosis*

Indicator	Year of graft	Infector	No. severe vein chlorosis / No. of plants grafted	Severe vein chlorosis in check tests
Norfolk Giant	1945	Norfolk Giant	5/7	—
Norfolk Giant	1945	St Walfried	7/12	—
Baumforth's B	1945, 48	Norfolk Giant	0/11	0/3
Lloyd George (N.Z.)	1947, 48	Norfolk Giant	9/10	1/1
Lloyd George (Comm.)	1945, 48	Norfolk Giant	8/8	—
St Walfried	1945, 48	Norfolk Giant	8/14	0/1
Malling Enterprise	1948	Norfolk Giant	0/3	—
Malling Jewel	1948	Norfolk Giant	0/3	—
Malling Landmark	1947, 48	Norfolk Giant	4/4	4/4
Malling Seedling M	1947, 48	Norfolk Giant	2/4	—
Malling Notable	1948	Norfolk Giant	2/3	—
Malling Promise	1947, 48	Norfolk Giant	3/6	3/3
Newburgh	1947, 48	Norfolk Giant	0/4	0/3
Cuthbert	1947, 48	Norfolk Giant	0/2	—

Vein chlorosis and down-curling of leaves, in addition to mosaic symptoms caused by leaf mottle and mild yellows viruses, developed on eight of fourteen healthy St Walfried plants grafted to Norfolk Giant infectors. Four samples of St Walfried showing vein chlorosis were selected from commercial stocks in 1944, and seven out of twelve canes produced severe vein chlorosis symptoms on Norfolk Giant.

The results of grafting Norfolk Giant infectors to plants of other varieties are given in Table 5. Conspicuous vein chlorosis and dwarfing of canes were observed on plants of Malling Notable, Malling Promise and Malling Seedling M. Symptoms on Malling Promise were, characteristically, restricted to the leaf margins and the leaves were down-curled (Pl. 4, fig. 8). Four plants of Malling Landmark grafted in 1947 and 1948 developed severe chlorotic blotching and vein chlorosis, and the young canes were much stunted. All four proved to be infected with severe vein chlorosis.

Despite repeated attempts, severe vein chlorosis has not been transmitted to Baumforth's Seedling B or the North American varieties Newburgh and Cuthbert.

DISCUSSION

Exclusive reliance on grafting as a mode of transmission leads to obvious difficulties in the interpretation of results. Strictly speaking, claim can only be made for the differentiation of three diseases on the basis of the symptoms they cause on Norfolk Giant. Even if, as is presumed, the diseases are caused by three individual viruses, none of these could have been transmitted unaccompanied by other viruses known

or not known to be present in the infectors. For reasons already discussed (Cadman, 1951), cross-protection tests provide the only guide to relationships between raspberry viruses, and these are not infallible.

The present evidence is considered to favour strain relationship between the three vein chlorosis viruses for the following reasons. First, all 'healthy' plants of Baumforth's B appear to be infected with mild vein chlorosis, and severe vein chlorosis has never been transmitted successfully to this variety. Secondly, it has been shown that, whereas healthy plants of Norfolk Giant developed typical symptoms, plants of Baumforth's B showed nothing other than mild vein chlorosis when grafted to Malling Landmark plants infected with moderate vein chlorosis. On the other hand, 'healthy' plants of Baumforth's B developed curly dwarf disease when grafted to plants of Lloyd George and Norfolk Giant showing moderate vein chlorosis symptoms. The last result is most simply explained by postulating first, that curly dwarf is caused by a virus etiologically distinct from the three vein chlorosis viruses and, secondly, that it was present in the particular Lloyd George and Norfolk Giant infectors used.

The mild and moderate vein chlorosis diseases are probably of negligible importance in the field. Although the second of these is widespread among commercial stocks of a great many varieties, infected plants are not noticeably less vigorous than uninfected ones and symptoms are not modified by the presence of group II viruses. Combinations of moderate vein chlorosis with other group I viruses, on the other hand, depress vigour proportionately to the amount of chlorosis they induce. If the results of infection with severe vein chlorosis can be considered as due solely to one virus, leaf symptoms are accompanied by marked stunting of cane growth. A disease of this type is commonly responsible for loss of vigour in stocks of Lloyd George, Malling Notable and Malling Seedling M in Scotland, but does not appear to be widespread there in other varieties. As leaf symptoms are expressed by the majority of present-day raspberry varieties on infection with the vein chlorosis viruses, roguing should prove an effective means of control.

Thanks are due to Dr A. R. Hill for his help with the aphid experiments, and also to Dr R. V. Harris and Dr I. W. Prentice for their advice and help during the course of this work. Acknowledgement is also made to the many growers who supplied experimental material and to the Agricultural Research Council who financed the work of which this forms a part.

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EXPLANATION OF PLATES 3 AND 4

PLATE 3

Figs. 1–3. Symptoms, on Norfolk Giant, of mild, moderate and severe vein chlorosis respectively.
Fig. 4. Mild vein chlorosis symptoms on leaf from 'healthy' plant of Baumforth's Seedling B.

PLATE 4

Fig. 5. Moderate vein chlorosis symptoms on Malling Promise.
Fig. 6. Symptoms of leaf mottle and moderate vein chlorosis on Malling Landmark.
Figs. 7 and 8. Severe vein chlorosis symptoms on Lloyd George and Malling Promise, respectively.

Figs. 1 and 8 are reproduced by kind permission of the editor of *Scientific Horticulture.*

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Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

STUDIES IN *RUBUS* VIRUS DISEASES

III. A VEINBANDING DISEASE OF RASPBERRIES

By C. H. CADMAN*

Scottish Raspberry Investigation (East Malling Research Station), University College, Dundee

(With Plate 5)

A chlorotic veinbanding disease of raspberries is shown to be due to a virus transmissible by the aphid *Amphorophora rubi* Kalt. after infection feeding periods of 18 hr. or more.

This virus causes leaf symptoms, masked in hot weather, on a wide range of European and North American varieties. Symptoms on the latter are analogous to those of the red raspberry mosaic of American authors.

The names veinbanding disease and raspberry veinbanding virus are proposed.

Symptoms of mosaic disease of the European red raspberry were considered by Harris (1940) to be of two types which he designated mosaic 1 and mosaic 2. Mosaic 1 symptoms were diffuse interveinal chlorotic patches accompanied by symmetrical down-curling of the lamina about the mid-rib, and these were masked on foliage produced under hot summer conditions. The disease was graft-transmitted from Baumforth's Seedling B and Lloyd George to mosaic-free plants of these varieties. It was found affecting only a few varieties and appeared to be caused by one virus.

A disease found on plants of the Norfolk Giant variety in eastern Scotland in 1944 proved to be graft-transmissible and to produce symptoms on Baumforth's B and Lloyd George similar to those of mosaic 1 disease. The experiments here reported show that the causal virus is transmissible by the aphid *Amphorophora rubi* Kalt. and induces mosaic symptoms on a wide range of raspberry varieties. The symptoms produced on certain North American varieties are analogous to those of North American red raspberry mosaic.

The techniques used were described in an earlier paper (Cadman, 1951).

Experimental results

Transmission by grafting

The infectors were mainly plants of Norfolk Giant, NG 6, selected from a commercial stock in 1944. These were supplemented by plants graft-infected from NG 6 and by other samples from commercial stocks of Norfolk Giant and St Walfried, considered, from the results of graft tests, to have the same disease.

* Now at Scottish Horticultural Research Institute, Mylnefield, Invergowrie, by Dundee.

The virus content of stocks of indicator varieties used in the early experiments was largely unknown. Latterly, indicator plants of known virus content have been used and some of the positive transmissions checked by back grafts to Norfolk Giant (L 234 stock) or Lloyd George (New Zealand stock). The results of the grafting experiments are summarized in Table 1.

Norfolk Giant

On two plants of Norfolk Giant (L 234 stock), grafted in 1945 to NG 6, pronounced chlorosis of the main leaf veins appeared on foliage of the fruiting canes in May 1946 and on the young canes in late June. This initial phase gave place to conspicuous yellow-green chlorotic bands bordering the main veins and enlarging into fan-shaped patches running out to the leaf margins (Pl. 5, fig. 1). Distribution of symptoms was often asymmetric, only the leaves on one sector of a cane or the leaflets of one half of a leaf being affected. Severely chlorotic leaves were slightly down-curled. These plants were grown on and, each year, developed symptoms in late May or early June but not on foliage produced during hot, sunny weather. In all these features the graft-infected plants were indistinguishable from those of the field sample NG 6.

Baumforth's Seedling B

Two plants of Baumforth's B were successfully graft-infected in 1945 and a third in 1948 and these first showed symptoms in June 1946 and late May 1949 respectively. The first sign of infection was a localized accentuation of the mild vein chlorosis symptoms present on all 'healthy' plants of Baumforth's B used (Cadman, 1952). Leaves produced later in the season by the young canes developed conspicuous interveinal chlorosis and were markedly down-curled. Masking of symptoms on all three plants was noted during sunny weather in 1947 and 1949.

The disease on these plants corresponded in all respects with the mosaic 1 disease of Baumforth's B described by Harris (1933, 1940).

Lloyd George

Although the Lloyd George plants used by Harris in 1932 (Harris, 1940) are presumed to have been virus-free, commercial stocks of this variety free from visual symptoms have been shown to be extensively virus infected (Prentice & Harris, 1950). A healthy stock, propagated from plants imported from New Zealand and found to be infected only with an unidentified latent virus (Cadman, unpublished), has only recently become available, and experiments with this stock will be described first.

In 1947, four plants and, in 1948, five plants of Lloyd George (New Zealand stock) were grafted with infected Norfolk Giant scions. Symptoms similar to those described on Norfolk Giant and Baumforth's B developed on all these plants (Pl. 5, fig. 2) and symptoms were masked in hot weather. Cane growth on the infected plants was less vigorous than on ungrafted control plants.

Plants of a vigorous, though virus-infected, commercial stock of Lloyd George grafted in 1945, 1947 and 1948 to infected Norfolk Giant showed veinbanding symptoms coupled with leaf distortion, much additional chlorosis and dwarfing of canes. This intensification of symptoms was thought to be due to viruses initially present in the indicator plants, which included those of mild yellows (Cadman & Harris, 1951), vein chlorosis (Cadman, 1952) and mosaic 2. Harris (1940) distinguished two types of mosaic symptoms on Lloyd George designated *b* and *c*. Type *b* symptoms were similar to those that developed on the New Zealand stock and were attributed to mosaic 1 alone. Type *c* symptoms were characterized by irregular leaf distortion associated with irregularly shaped and distributed, deeply depressed, chlorotic areas, and were attributed to joint infection with the causes of mosaic 1 and 2 diseases.

Other European varieties

The results of experiments with other varieties are shown in Table 1. When infected by grafting, plants of Burnetholm Seedling, St Walfried and the Malling varieties Enterprise, Jewel, Notable and Seedling M showed veinbanding symptoms

TABLE 1. *Results of graft transmissions of veinbanding*

Indicator	Year of graft	Infector	No. veinbanding / No. of plants grafted	Veinbanding in check tests
Norfolk Giant	1945	Norfolk Giant	2/2	—
Baumforth's B	1945	Norfolk Giant	2/6	—
Baumforth's B	1948	Norfolk Giant	1/2	—
Lloyd George (N.Z.)	1947	Norfolk Giant	4/4	0/2
Lloyd George (N.Z.)	1948	Norfolk Giant	5/5	1/5
Lloyd George (N.Z.)	1947	Red Cross	2/2	—
Lloyd George (N.Z.)	1949	Burnetholm Seedling	3/3	—
Lloyd George (Comm.)	1945	Norfolk Giant	1/1	—
Lloyd George (Comm.)	1947	Norfolk Giant	1/1	—
Lloyd George (Comm.)	1948	Norfolk Giant	1/5	—
Lloyd George (Comm.)	1945	St Walfried	6/10	—
St Walfried	1945	Norfolk Giant	1/1	—
St Walfried	1948	Norfolk Giant	2/5	2/4
St Walfried	1945	St Walfried	4/7	—
Burnetholm Seedling	1947, 48	Norfolk Giant	3/4	0/1
Malling Enterprise	1948	Norfolk Giant	2/3	—
Malling Jewel	1947, 48	Norfolk Giant	2/4	—
Malling Landmark	1947	Norfolk Giant	2/2	—
Malling Landmark	1948	Malling Promise	3/3	—
Malling Seedling M	1947, 48	Norfolk Giant	4/4	—
Malling Notable	1947, 48	Norfolk Giant	4/4	—
Malling Promise	1947, 48	Norfolk Giant	8/8	—
Malling Exploit	1949	Norfolk Giant	5/5	—
Malling Seedling Z	1949	Norfolk Giant	5/5	—
Newburgh	1947, 48	Norfolk Giant	3/4	1
Cuthbert	1947, 48	Norfolk Giant	2/2	—
Herbert	1947	Norfolk Giant	1/1	—
Latham	1949	Norfolk Giant	3/5	—
Viking	1947, 49	Norfolk Giant	5/6	—
Rubus saxatilis	1948	Norfolk Giant	3/5	—
R. occidentalis × *R. idaeus*	1950	Lloyd George	5/5	—

similar to those described on Norfolk Giant. Plants of St Walfried and Malling Landmark developed interveinal chlorotic blotching in addition to veinbanding, due, presumably, to the leaf mottle virus in the infectors (Cadman, 1951).

Plants showing veinbanding symptoms have been found in commercial stocks of all the above varieties and scions from affected plants of Burnetholm Seedling, St Walfried and Red Cross produced typical veinbanding symptoms on Lloyd George. Whether this was the mosaic disease of Red Cross referred to by Harris (1940) is uncertain as he obtained no unequivocal transmissions of mosaic 1 from this variety to either Baumforth's B or Lloyd George and detected only mosaic 2 disease in the source plants. Graft tests in 1945 showed that the Red Cross infectors referred to in Table 1 were mosaic 2-infected. Transmission of a mosaic disease additional to mosaic 2 from Red Cross to plants of Malling Landmark and Malling Promise was recorded by Prentice & Harris (1950) but the symptoms were not described.

Malling Promise, Malling Exploit and Malling Seedling Z, varieties all selected from the progeny of a cross with the American variety Newburgh, showed symptoms similar to those on Newburgh, as described below (Pl. 5, fig. 3).

North American varieties

Leaf symptoms on three plants of Newburgh, graft-infected from Norfolk Giant and Malling Promise, resembled those on Lloyd George and other varieties, but the chlorosis tended to be fainter, more diffuse and irregular, and restricted mainly to the leaf margins. Symptoms were completely masked on leaves produced by all three plants during warm sunny weather.

Plants of Cuthbert, Herbert, Viking and Latham, initially free from mosaic but of unknown virus content, all showed veinbanding symptoms the year after graft inoculation. Leaf symptoms varied from one variety to another but were, in general, similar to those observed on Newburgh and European varieties.

Plants affected with a mosaic disease, tentatively identified by Dr G. H. Berkeley as American red raspberry mosaic, were found in stocks of the Chief, Latham and Viking varieties kindly supplied in 1949 by Dr A. W. S. Hunter of the Division of Horticulture of the Canadian Department of Agriculture. The symptoms on Latham and Viking resembled those on the graft-inoculated plants described above. Five plants of Lloyd George (New Zealand stock) were grafted in 1949 with scions from the mosaic-infected Canadian plants of Chief and Latham in June 1950 and all developed severe veinbanding symptoms, which resembled, but were more severe than, those on Lloyd George infected from NG 6. The two plants grafted with infected Latham became necrotic. It is, however, likely that the Canadian plants were infected with viruses additional to that causing the veinbanding symptoms.

Rubus *species*

Of five plants of *Rubus saxatilis* grafted in 1948 to infected Norfolk Giant, three developed severe interveinal chlorosis, leaf curling and necrosis of the stolon tips. Two of these plants were dead by spring 1949; the third was extremely dwarfed and produced only a few curled chlorotic leaves.

Acute symptoms consisting of leaf blotching and necrosis of the cane tips developed on five plants of a 'purple raspberry' seedling (*R. occidentalis* × *R. idaeus*) 4 weeks after grafting, in May 1950, with infected Lloyd George scions. Leaves subsequently produced showed only slight interveinal chlorotic spots. These are not considered significant, as similar symptoms showed on the five ungrafted controls whilst the grafted plants developed typical veinbanding symptoms in late August 1950.

TABLE 2. *Results of transmission experiments with* Amphorophora rubi *and Lloyd George infectors*

Year	Indicator	Infection feeding period	Aphids per plant	Test feeding period (days)	No. of infections
1950	*R. idaeus* × *R. occidentalis*	18 hr.	20	2	3/5
1949	Lloyd George	24 hr.	15	2	3/20
1947	Norfolk Giant	18 days	∞	3	1/10
1949	*R. saxatilis*	38 days	5	3	3/5

Transmission by aphids

In 1947, large numbers of *Amphorophora rubi* Kalt. from a culture reared on Norfolk Giant (L234) plants were allowed to feed for 18 days on a plant of Red Cross showing veinbanding symptoms. About 1000 aphids were then transferred to each of ten healthy plants of Norfolk Giant on which they were left for 2 days. Some weeks later one of the Norfolk Giant plants developed veinbanding symptoms. This result was confirmed by experiments in 1949 and 1950, using the New Zealand Lloyd George plants infected from NG 6 as infectors and Lloyd George, *Rubus saxatilis* and a purple raspberry seedling (*R. occidentalis* × *R. idaeus*) as indicators. The results (Table 2) showed that the veinbanding virus is transmitted after 18 hr. or more feeding on the infectors. The symptoms on *R. saxatilis* were less severe than those on plants infected by graft from Norfolk Giant and the diagnosis was not checked by back grafting to a *R. idaeus* host. All the purple hybrid plants produced symptoms indistinguishable from those observed on this host in the grafting experiments.

Experiments were made in 1949 and 1950 with *R. occidentalis* var. Cumberland as an indicator. Acute symptoms, consisting of conspicuous leaf blotching and necrosis of stem tips, developed on a proportion of all plants (Table 3) that received *Amphorophora rubi* fed for periods varying from $\frac{1}{2}$ to 48 hr. on infected Lloyd

George (initially New Zealand stock). The chronic symptoms that subsequently developed consisted of chlorosis, indistinct leaf blotching, rosetting of cane tips and dwarfing of canes. They were not clearly distinguishable from those caused by the leaf mottle virus (Cadman, 1951) or by another, unidentified, latent virus present in all plants of the New Zealand Lloyd George stock (Cadman, unpublished). As the infectors used in these experiments were graft-infected from NG 6 containing both veinbanding and leaf mottle viruses, they were probably infected with both of these in addition to the latent virus. It would therefore seem that two or more components of this mixture were transmitted by *A. rubi* on each occasion. Scions from the infected Cumberland plants repeatedly failed to unite with red raspberry (*Rubus idaeus*) plants so that the results could not be checked by graft tests.

TABLE 3. *Results of transmission experiments with* Amphorophora rubi *using Lloyd George infectors and* Rubus occidentalis *var. Cumberland indicators*

(Aphids per plant: various; I.F.P. various; T.F.P. constant 48 hr.)

Year	No. of aphids per plant	Infection feeding period: 6 min.*	10 min.*	½ hr.	1 hr.	2 hr.	4 hr.	8 hr.	16 hr.	24 hr.	48 hr.	Control
1949	5	—	—	0/5	0/5	2/5	0/5	1/5	1/5	0/5	4/5	0/5
1949	14	—	—	4/5	3/5	0/5	0/5	4/5	5/5	3/5	—	0/5
1949	14	—	—	—	—	4/5	4/5	—	—	—	—	0/5
1950	20	0/9	0/5	1/10	—	—	—	—	—	—	—	—
		0/9	0/5	5/20	3/10	6/15	4/15	5/10	6/10	3/10	4/5	0/15

* Aphids pre-starved for 4–8 hr.

TABLE 4. *Results* (1951) *of serial transfer experiment, August* 1950, *with* Amphorophora rubi, *NG* 6 *infectors and* Rubus occidentalis *var. Cumberland indicators*

(Aphids per plant: 20*; I.F.P. constant, 20 hr.; T.F.P. various.)

Transfer	1st	2nd	3rd	4th
Test feeding period	1 hr.	2 hr.	3 hr.	12 hr.
Total no. of plants infected	4/5	3/5	1/5	0/5
No. showing veinbanding symptoms	2/5	2/5	1/5	0/5
No. showing complex symptoms	2/5	1/5	0/5	0/5

* Twenty aphids were transferred from the infector to each indicator of the first transfer set. Small numbers of aphids died or were lost in the course of the subsequent transfers.

In a serial transfer experiment with *Amphorophora rubi* made in August 1950 (Table 4), the aphids were first fed for 20 hr. on NG 6 infectors and then given transfer feeds of 1, 2, 3 and 12 hr. respectively on healthy Cumberland indicators. Leaf blotching and necrosis developed in the current season on two plants of the first transfer set, and one of these plants subsequently died. In spring 1951, veinbanding symptoms (Pl. 5, fig. 4) developed on plants of the 1st, 2nd and 3rd transfer sets. Plants of the 4th transfer set and five control plants that received aphids from

the stock culture showed no symptoms. It was therefore concluded that the veinbanding and leaf mottle diseases of NG 6 had been separated. The results, though scanty, suggest that veinbanding is caused by a virus more persistent than the leaf mottle virus, and that both viruses persist for only a short time in the aphid. This last finding is supported by earlier data (Cadman, 1951) and by the results of an experiment in 1949 in which *A. rubi* fed for 16 hr. on Lloyd George veinbanding infectors failed to induce symptoms on the Cumberland indicators after the initial 24 hr. test-feeding period.

Conclusions and discussion

It is concluded that the veinbanding disease identified on Norfolk Giant and other varieties in Scotland is identical with the mosaic 1 disease described by Harris (1940) and the data agree with that author's supposition that the symptoms are caused by only one virus. As the name mosaic 1 denoted a disease category rather than a specific disease, the name veinbanding for the disease and virus respectively is now proposed.

The disease is one of the commonest and most conspicuous mosaic diseases of raspberries in Britain. It affects many different varieties, causing severe chlorosis and loss of vigour, and the leaf symptoms are readily masked when temperature and light intensity are high. The virus is transmissible by *A. rubi* Kalt. after an infection feed of 18 hr. or less and probably persists in the vector for not more than 24 hr.

The symptoms caused by the veinbanding virus on North American red and black raspberry varieties resemble those of analogous diseases of these hosts described by workers in the United States of America and Canada. Leaf symptoms, closely similar to those described above and said to be due to infection with red raspberry mosaic, are illustrated by Rankin (1927) and Zeller & Schuh (1944). Some confusion exists in the literature regarding the identity of this disease. Bennett (1932) used the name red raspberry mosaic for diseases transmissible by *A. rubi* and varying in expression on red raspberry varieties from mild mottling to severe chlorosis, these symptoms being masked during periods of high temperature. He pointed out the difficulty of distinguishing discrete types of symptoms on red raspberries, a difficulty also referred to by Harris (1935), but defined three arbitrary grades of virulence of red raspberry mosaic on the basis of the reactions of the Cumberland black raspberry to infection. These he styled mild, medium severe and severe red raspberry mosaic, the symptoms on Cumberland being leaf mottling or mottling plus necrosis of the petioles and stems. The symptoms on red raspberries illustrated by Rankin (1927) and Zeller & Schuh (1944) apparently correspond with Bennett's definition of severe mosaic.

Bennett (1932) stressed the lack of critical data capable of deciding the question whether these differences in symptoms were due to different viruses or to strains of one virus. Cooley (1936), with fewer scruples, ascribed 'all types of mosaic of raspberry other than the distinct cases known to be due to yellow mosaic virus' to

one virus which he named virus 1. This he regarded as synonymous with Rankin's (1931) mosaic viruses 1 and 3 and presumed to be the cause of Bennett's (1932) three grades of red raspberry mosaic, changing the name to green mottle mosaic. Cooley's virus 1 became *rubus* virus 1 in Smith's (1937) catalogue and *Marmor rubi* of Holmes (1939). The implications of these changes in nomenclature appear unjustifiable in the light of Bennett's findings, and the veinbanding virus cannot at present be identified with Cooley's virus 1 or with any form of red raspberry mosaic. The results reported in the present paper, in suggesting relationships between the raspberry viruses endemic to North America and Britain, point to a need for further investigation of this field.

Evidence has been obtained that the Cumberland variety of *Rubus occidentalis*, though extremely susceptible to infection by means of *Amphorophora rubi*, does not show different initial symptoms upon infection with the different viruses found to be transmissible by this aphid. These include the latent virus of New Zealand Lloyd George, leaf mottle, mosaic 2 and yellows viruses, none of which has been found regularly to persist in the vector for more than 24 hr. (Cadman, unpublished). The interpretation to be placed on these results is, however, at present uncertain. Without exception, all the infectors used in these experiments contained two or more viruses and one of them, for example leaf mottle or the latent mosaic virus referred to, may have been common to all. The apparent lack of differential reactions in the Cumberland indicators may, therefore, be due to the design of the experiments, the infection and test feeding periods being such that, on each occasion, the vector picked up and transmitted the leaf mottle component in addition to the other virus or viruses present in the infector.

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Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

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EXPLANATION OF PLATE 5

Figs. 1–3. Veinbanding symptoms on Norfolk Giant, Lloyd George (New Zealand stock), and Newburgh, respectively.
Fig. 4. Leaf from Cumberland black raspberry plant (*Rubus occidentalis*) infected with veinbanding by means of *Amphorophora rubi.*

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STUDIES ON THE APHID TRANSMISSION OF A STRAIN OF HENBANE MOSAIC VIRUS

By R. H. E. BRADLEY*

Rothamsted Experimental Station, Harpenden, Herts

(With 1 Text-figure)

A virus causing a wilt of *Datura stramonium* was identified as a strain of henbane mosaic virus. It causes necrotic local lesions in *Nicotiana rustica*, and local lesions are demonstrable in tobacco by staining with iodine. Some of the factors affecting its transmission by *Myzus persicae* (Sulz.) were studied quantitatively using these lesions.

Infective aphids differed little in their ability to cause infection, and usually produced two or three lesions. The duration of the feeding puncture did not affect the number of infections and had little effect on the percentage of aphids becoming infective. Transmissible virus did not seem to be continually imbibed while aphids fed on infected plants, and there were indications that it was acquired immediately before aphids withdrew their stylets from the leaf. Aphids became infective when allowed to make feeding punctures into epidermis stripped from infected leaves.

M. persicae transmitted during feeding punctures as brief as 5–10 sec.; the probability of single feeding punctures resulting in infection reached a maximum with those lasting from 20 to 30 sec., during which the stylets did not penetrate as far as the centre of the epidermal cell and little or no saliva appeared to be ejected. *M. persicae* did not transmit the virus when its stylets were artificially wetted with infective sap.

Periods of darkness before inoculation with datura wilt virus increased the susceptibility of *Nicotiana rustica* to infection by rubbing, but not to infection by aphids.

A virus found causing a severe wilt in *Datura stramonium* growing at Oxford in 1948 was first thought to be unrelated to any previously described and was called datura wilt virus (DWV). Subsequent tests identified it as a strain of henbane mosaic virus, first described by Hamilton (1932) as hyoscyamus 3 virus, and later recorded in *Atropa belladonna* by Smith (1945). Dr Kenneth M. Smith kindly supplied a derivative of Hamilton's culture and the strain he obtained from *A. belladonna*, and the properties of these were compared with those of DWV.

The results of serological and cross-protection tests showed that the three were related. Rabbits injected with sap from plants infected with each produced antisera which specifically precipitated either of the others. Also systemic infection with the virus from *A. belladonna* protected *Nicotiana tabacum* against invasion by either hyoscyamus 3 virus or DWV.

* Now in the Division of Entomology, Fredericton, N.B., Canada.

The symptoms caused by the three strains in *N. tabacum* (var. White Burley), *N. glutinosa*, *Hyoscyamus niger* and *Datura stramonium* were of the same type as those described by Hamilton (1932), except that those caused by DWV were more severe and those by the strain from *Atropa belladonna* were less severe. All three strains caused discrete necrotic local lesions in *Nicotiana rustica*.

The properties of the three strains were compared using sap from infected *N. tabacum* and all behaved similarly. The dilution end-point was between 10^{-4} and 10^{-5}; the thermal inactivation-point for 10 min. heating was between 55 and 60° C.; sap kept at 21° C. was infective for 4 weeks, but most of the infectivity disappeared during the first 4 days. These results agree with those reported by Watson (1938), except that she found longevity *in vitro* to be less than 6 days.

The three strains were transmitted by *Myzus persicae* Sulz. in the manner described by Watson (1938). The percentage of infective aphids was increased by a preliminary fasting period provided the insects fed on the infected plant for only a few minutes. The time for which they remained infective depended upon whether or not they were fed. If starved they remained infective for periods up to 12 hr., but if fed they lost their infectivity within minutes.

The present paper records further experiments showing the manner in which the feeding behaviour of aphids affects their efficiency as vectors.

Methods

Aphids were handled by methods similar to those described by Watson (1936). They were tested for their infectivity on either *Nicotiana rustica* or *N. tabacum* in the 5- to 7-leaf stage. *N. rustica* produced necrotic local lesions. In tobacco local lesions could be distinguished by applying Holmes's (1931) starch-iodine technique; the most consistent results were obtained by collecting leaves during the day, when they stained blue-black and the lesions appeared as lighter spots on a dark background. The number of lesions produced was roughly proportional to the number of infective aphids. For example, fasted aphids given a short infection feed and then moved to healthy tobacco leaves singly, in twos or fours, produced 21, 49 and 93 lesions respectively on thirteen leaves. Eight of the thirteen leaves colonized with single aphids developed lesions, suggesting that over 60% of the aphids were infective, a result similar to that obtained under comparable conditions when aphids are tested by their ability to cause systemic infections. For testing the infectivity of individual aphids on either *N. rustica* or *N. tabacum*, only one aphid was placed on each test leaf. *Myzus persicae* transmitting DWV usually loses its infectivity within an hour of leaving infected plants, and aphids were watched during the first hour of the test-feeding period and prevented from leaving the test leaves.

In preliminary experiments young systemically infected tobacco leaves were used as sources of virus, but the leaf hairs often hindered the aphid's movements and sometimes prevented them from feeding readily. By contrast, aphids quickly start to feed on the older leaves, which have fewer hairs. These smoother leaves seldom

become systemically infected, but when dusted with celite and rubbed with infective sap they develop many diffuse chlorotic lesions. Two weeks after inoculation, sap from these leaves has the same serological titre as sap from systemically infected leaves. Experiments showed that aphids became infective from such leaves as readily as by feeding on systemically infected leaves, and such inoculated leaves were therefore generally used for infection feedings. It is generally assumed that when aphids, particularly if they have been fasted, are placed on a leaf they soon settle down and feed continuously. However, this is not so. After fasting, an aphid usually probes the leaf several times with its proboscis, each probe occupying less than a minute. Whether or not aphids feed in the sense that liquids are taken into the stomach during this short time, is not known. These short periods will be referred to as 'feeding punctures'.

Experimental

Acquisition of datura wilt virus by Myzus persicae

An experiment was made to determine how the duration of the feeding puncture affects the acquisition of DWV. After 4 hr. preliminary fasting, each aphid was observed while it made one feeding puncture on an infected leaf, and the duration of the feeding puncture recorded as lasting from 0 to 5, 5 to 10, 10 to 20, 20 to 30, or over 30 sec. Each aphid was then tested overnight on a *Nicotiana rustica* leaf. About ten aphids were used in each category in each experiment. To get ten aphids in the 5–10 sec. treatment, most had to be disturbed by touching them with a camel-hair brush and this treatment is therefore not strictly comparable with the others.

Table 1. *Part played by the duration of feeding puncture of* Myzus persicae *in the acquisition of datura wilt virus*

	Time in seconds *M. persicae's* proboscis remained touching infected leaf				
	0–5	5–10	10–20	20–30	Over 30
Aphids tested	13	100	100	100	44
Aphids infective	0	17	72	63	25
Percentage infective	0	17·0	72·0	63·0	51·5
Lesions	0	36	178	175	61
Lesions per infective aphid	0	2·1	2·5	2·8	2·4

The results of ten such experiments (Table 1) show that no aphid making a feeding puncture of less than 5 sec. was infective, and that feeding punctures of 10–20 sec. produced the highest percentage of infective aphids. Similar results have been obtained by Sylvester (1949*b*, 1950*b*) with *Myzus persicae* transmitting sugar-beet mosaic virus and the *Brassica nigra* virus. Table 1 also shows that the number of infections produced per infective aphid is little affected by the duration of the feeding puncture.

To see whether disturbing the aphids used in the 5–10 sec. treatment was likely to have affected their vector efficiency, two experiments were made to test the

effects of such disturbances. In the first, aphids fasted for 4 hr. were allowed to make one feeding puncture on a healthy leaf before making one on an infected leaf. Half the aphids were disturbed after the puncture on the healthy leaf had been maintained from 10 to 30 sec. and half the aphids were allowed to complete this feeding puncture undisturbed. Each aphid was then allowed to make one feeding puncture on an infected leaf before being transferred to a healthy *Nicotiana rustica* leaf. The disturbing had no effect, for of thirty-five aphids used for each treatment, twenty-four of those disturbed during the feeding puncture on a healthy leaf became infective, compared with twenty-three not disturbed; these caused a total of thirty-eight and forty-two lesions respectively. After a period of preliminary fasting, over 85% of the aphids which maintained their initial feeding punctures for over 10 sec. withdrew their stylets during the succeeding 20 sec. It was observed that when an aphid was about to withdraw its mouthparts, it usually first waved its antennae; thus by disturbing aphids after the feeding puncture had been maintained from 10 to 30 sec. or when the antennae began to wave, aphids could be made to withdraw their stylets a few seconds prematurely. In the second experiment, aphids fasted for 1 hr. were allowed to make one feeding puncture on an infected leaf undisturbed and compared with aphids disturbed so that they withdrew their stylets prematurely. Disturbing aphids a few seconds before they would normally withdraw their stylets from infected leaves, reduced those that became infective from 66 to 33%, but did not affect the number of lesions produced per infective aphid. These results suggest that disturbing *Myzus persicae* during the infection-feeding puncture either causes some to withdraw their stylets before transmissible virus is acquired or, having acquired virus, to lose it when disturbed. As disturbing during test-feeding does not reduce the percentage of infective aphids (Table 8) loss of virus seems unlikely, and it appears that disturbing during the infection-feeding puncture causes the stylets to be withdrawn before virus is acquired. If this is so, many aphids appear to acquire the virus they can later transmit during the final few seconds of their feeding puncture. Disturbing the aphids in the 5–10 sec. treatment (Table 1) probably reduced the number of infective aphids in this group, and such brief feeding punctures may be more effective than the results in Table 1 suggest.

Over 60% of the fasted aphids used in these experiments became infective when given one infection-feeding puncture, and increasing the number of punctures did not increase the percentage of infective aphids, or the average number of lesions produced per infective aphid. Sylvester (1950*b*) has described comparable results with *M. persicae* and sugar-beet mosaic virus. To see if fasted aphids acquire virus only during their initial feeding puncture, one group made a first feeding puncture on a healthy leaf and then a second on an infected leaf, while another group made a feeding puncture only on an infected leaf. Of forty aphids used in eight experiments, twenty-three of the first and thirty of the second group were infective; the aphids in the two groups caused a total of forty-two and sixty-seven lesions

respectively. The differences between treatments were not statistically significant, and it appears that fasted *M. persicae* acquire DWV as readily after an initial feeding puncture on a healthy leaf as when the initial feeding puncture is made on an infected leaf.

An additional experiment was made to test the effect of longer periods on a healthy leaf previous to the infection-feeding puncture. Fasted aphids were placed on a healthy or an infected tobacco leaf and observed until they made at least one feeding puncture. Some were moved to test plants immediately after completing their initial feeding puncture on the infected leaf and others were left on it for periods of 5, 10 or 15 min. Aphids were transferred from the healthy to the infected leaf after periods of about 4, 9 and 14 min., and were observed until they had completed one feeding puncture, when they were removed to test plants. The results (Table 2) of six such experiments show that the highest proportion of infective aphids occurred in the group that made the initial feeding puncture on

Table 2. *The effect on the acquisition of datura wilt virus of various periods on a healthy leaf previous to a feeding puncture on an infected leaf*

	Time on infected leaf				Time on healthy leaf plus one feeding puncture (about 1 min.) on infected leaf		
	1 feeding puncture	5 min.	10 min.	15 min.	5 min.	10 min.	15 min.
Aphids infective*	24	19	16	10	15	14	10
Lesions	53	44	28	12	26	22	15
Lesions per infective aphid	2·2	2·3	1·8	1·2	1·7	1·6	1·5

* Out of a total of thirty.

the infected leaf and were then removed, and that prolonging the period on the infected leaf reduced the proportion. Approximately the same number of infective aphids occurred in the groups that were first on a healthy leaf as in those that spent similar periods continuously on the infected leaf. The number of lesions produced per infective aphid decreased with increased time on plants before being transferred to the test leaves, but the infective aphids which spent most of their time on a healthy leaf produced as many lesions as those that spent all their time on the infected leaf. Over 70 % of the aphids first placed on the infected leaf must have become infective during the initial feeding puncture, and some of those removed after 5, 10 and 15 min. must have lost their ability to transmit during succeeding feeding punctures on the infected leaf.

Feeding puncture of Myzus persicae *in relation to infecting with datura wilt virus*

The work was extended to study the effect of the duration of the feeding puncture in causing an infection. After fasting for 4 hr. single aphids were allowed to make two or three feeding punctures on an infected leaf; the average duration of these feeding punctures was about 30 sec. The aphid was then transferred to a healthy

Nicotiana rustica or *N. tabacum* leaf and observed continuously with a hand-lens. Each time an aphid touched the leaf with its proboscis, the site and the duration of the feeding puncture were recorded. The sites were usually well separated, but to ensure this aphids had sometimes to be disturbed. The number of feeding punctures varied from three to eleven with different aphids; some aphids made a few punctures and then did not attempt to feed again. Each aphid was observed for approximately 40 min. From three to five aphids were tested on various days between November 1949 and March 1950.

The local lesions showed which of the feeding punctures had caused infections. In all, over 200 feeding punctures were observed and no lesions developed except where a puncture had been recorded. Of twenty-one aphids tested on *N. rustica* and nineteen on *N. tabacum*, fifteen caused infections in each host. With the infective aphids, 103 feeding punctures on *N. rustica* produced thirty-nine lesions, and 106 on *N. tabacum* produced thirty-eight lesions. There was no significant difference between the durations of feeding punctures on *N. rustica* and *N. tabacum*. As the two plants do not differ in their susceptibility to infection, in the following analysis the results of aphids tested on both hosts are treated together. Of 209 feeding punctures observed during approximately the first 40 min. of each aphid's test period, only forty-four lasted over a minute and only two over 5 min. (Table 3). Statistical analysis of the durations of the feeding punctures on both the infected and the test leaves showed no significant differences between infective and non-infective aphids. Nor was there anything in the behaviour of non-infective aphids to suggest why they failed to transmit.

The results in Table 3 show that infective aphids may make one or more feeding punctures without causing an infection. These failures do not appear to be due to feeding punctures of too short duration; aphids 4, 21 and 27 (Table 3) did not infect during feeding punctures lasting over a minute, but did infect during 20–30 sec. feeding punctures. There were no significant differences between the durations of infective and non-infective feeding punctures. To find what part the duration of a feeding puncture plays in causing an infection, those up to and including the last infection caused by each aphid shown in Table 3 are grouped in Table 4 according to their duration. The results show that 38 % of the feeding punctures lasting from 5 to 20 sec. caused infections and 68 % of those lasting over 20 sec. caused infections. There was no apparent difference between the effectiveness of the 5–10 and 10–20 sec. feeding punctures, and those lasting over 20 sec. up to 5 min. did not increase the probability that infection would occur.

Selecting those feeding punctures up to the last infection caused by each aphid may have biased the results in Table 4, but this seems unlikely because similar results are obtained if the durations of the first five feeding punctures of aphids 7 to 30 from Table 3 are grouped according to their duration.

Before making a feeding puncture, *Myzus persicae* often touches the leaf for an instant several times, as if searching for a suitable place to insert its stylets. During

Table 3. *The duration of feeding punctures of* Myzus persicae *transmitting datura wilt virus*

	Duration of successive feeding punctures on test leaves											
Aphid	1	2	3	4	5	6	7	8	9	10	11	Total
1	15	**50***	5	—	—	—	—	—	—	—	—	1/3
2	**60**	**15**	**105**	**360**	—	—	—	—	—	—	—	3/4
3	**5**	40	30	**90**	—	—	—	—	—	—	—	2/4
4	100	**30**	60	**20**	—	—	—	—	—	—	—	2/4
5	**30**	60	**60**	90	—	—	—	—	—	—	—	2/4
6	**30**	10	5	5	—	—	—	—	—	—	—	1/4
7	**20**	**20**	20	**20**	30	—	—	—	—	—	—	3/5
8	**40**	**20**	25	10	15	—	—	—	—	—	—	2/5
9	10	**15**	5	10	150	—	—	—	—	—	—	1/5
10	**20**	60	40	140	180	120	—	—	—	—	—	1/6
11	**30**	15	**40**	**35**	30	5	—	—	—	—	—	3/6
12	**15**	**10**	10	10	15	**30**	—	—	—	—	—	3/6
13	**120**	60	35	10	**5**	**60**	20	—	—	—	—	3/7
14	**40**	20	15	10	10	10	**20**	—	—	—	—	2/7
15	140	**150**	120	40	15	35	720	—	—	—	—	2/7
16	35	**20**	20	20	25	20	60	—	—	—	—	1/7
17	**25**	**15**	**70**	15	**50**	120	15	—	—	—	—	1/7
18	15	**30**	10	**30**	5	15	10	—	—	—	—	4/7
19	15	**5**	**10**	5	**15**	10	5	**5**	—	—	—	4/8
20	**15**	20	15	**15**	**30**	15	5	20	—	—	—	3/8
21	360	**20**	**40**	15	20	20	**20**	10	—	—	—	3/8
22	**600**	**15**	**20**	**20**	15	35	15	10	840	—	—	4/9
23	40	**30**	**30**	15	**35**	25	25	35	40	—	—	3/9
24	15	45	**55**	20	**15**	**30**	20	10	20	—	—	3/9
25	30	10	15	**40**	15	20	50	20	40	—	—	1/9
26	**30**	20	**120**	**10**	5	**20**	15	10	20	15	—	4/10
27	**20**	**20**	90	70	**25**	**10**	60	30	20	30	—	4/10
28	10	5	**20**	15	**15**	15	**15**	**10**	5	10	—	4/10
29	5	5	**10**	**5**	15	**50**	20	40	10	50	—	3/10
30	40	**20**	25	**20**	5	**20**	15	5	15	25	**15**	4/11
Total	16/30	16/30	12/30	12/29	8/24	7/21	3/18	2/12	0/9	0/5	1/1	77/209
% infective	55·3	53·3	40·0	41·4	33·3	33·3	16·7	16·7	·		—	36·8

* Figure in black indicates feeding puncture caused an infection.

Table 4. *Part played by the duration of feeding puncture of* Myzus persicae *in causing an infection with datura wilt virus*

	Duration of feeding punctures on test leaf							
Test leaf	5–10 sec.	10–20 sec.	20–30 sec.	30–40 sec.	40–60 sec.	1–5 min.	5–10 min.	Total
N. tabacum	3/8*	11/28	13/18	3/3	6/6	2/5	0/1	38/69
N. rustica	2/5	6/17	8/12	11/15	3/7	7/12	2/2	39/70
Total	5/13	17/45	21/30	14/18	9/13	9/17	2/3	77/139
% infective	38·5	37·7	70·0	77·8	69·2	58·8	66·6	55·4

* Denominator is number of feeding punctures observed and numerator is number that caused infections.

these experiments, sixty-one sites were recorded where an infective aphid touched the leaf in this manner. No infections occurred at any.

The totals in Table 3 show that the number of aphids causing infections decreases with successive feeding punctures. It is sometimes assumed that the infectivity of aphids, i.e. the number of infections they cause and the length of time they remain infective, is determined by the amount of virus they contain, but other factors seem equally important. Aphids nos. 10–30 in Table 3 inclusive made six or more feeding punctures on the test leaves and eleven caused infection after the fifth feeding puncture and ten did not. There were no differences between these two groups in the probabilities that one feeding puncture would cause an infection up to the fifth feeding puncture. The probability of infection from one feeding puncture between the first and fifth puncture time was the same (about 0·5) for both groups. If those aphids which caused infections after the fifth feeding puncture contained more virus than those that did not, and quantity of virus affects the probability of infection, the probability of infection during the first or second feeding puncture might reasonably be expected to be greatest with those aphids that remained infective longest. The results in Table 3, however, show no such correlation, and there is no reason to believe that the quantity of DWV in infective aphids varied greatly.

There were no significant differences between the durations of successive feeding punctures recorded in Table 3. If the aphids also did not differ in their infectivity, the infections should be binomially distributed. The distribution of infections caused by aphids nos. 7 to 30 in Table 3, during the first five feeding punctures of each, was tested. Of 120 feeding punctures made by twenty-four aphids, fifty-three caused infections; therefore, the probability of one feeding puncture causing infection was $p = 53/120 = 0{\cdot}44$. During five feeding punctures an aphid may cause 0, 1, 2, 3, 4 or 5 infections. If infections are binomially distributed, the probabilities for 0, 1, 2, 3, 4 or 5 infections occurring are q^5, $5q^4p$, $10q^3p^2$, $10q^2p^3$, $5qp^4$ and p^5 respectively (expansion of the binomial $(p+q)^5$, where p is the probability of one feeding puncture causing infection and $q = 1-p$, the probability that a feeding puncture will not cause infection). By substituting $p = 0{\cdot}44$ and $q = 0{\cdot}56$ in these probabilities and multiplying by 24, the expected frequency of 0, 1, 2, 3, 4 and 5 infections for the last twenty-four aphids in Table 3 are obtained. The calculated frequencies do not differ significantly from the observed (Table 5), strongly suggesting that the aphids had similar abilities to cause infection. Smith & Lea (1946), working with *M. persicae* and tobacco rosette virus, found that infections were not binomially distributed and they concluded that, though subjected as far as possible to similar treatment, the aphids were not equally infective. Tobacco rosette is a persistent virus and may have a more complex relation with its insect vector than the non-persistent DWV.

When aphids are given a short infection feed, more become infective if they have previously fasted than if they have been feeding. This phenomenon was first

described by Watson (1938) with hyoscyamus virus 3 and has since been noted with many viruses. To see whether fasting affected the feeding behaviour of aphids, the duration of feeding punctures made by fasted and non-fasted aphids were measured.

Table 5. *Distribution of infections caused by aphids 7 to 30 (Table 3) during the first five feeding punctures*

$$p=\frac{53}{120}=0{\cdot}44;\ q=0{\cdot}56.$$

	Infections per aphid	Expected	Actual
$p_0=q^5=0{\cdot}055$	0 or 1	6·50	6
$p_1=5q^4p=0{\cdot}216$			
$p_2=10q^3p^2=0{\cdot}341$	2	8·18	9
$p_3=10q^2p^3=0{\cdot}267$	3	6·41	7
$p_4=5qp^4=0{\cdot}104$	4 or 5	2·88	2
$p_5=p^5=0{\cdot}016$	Total	23·97	24

Aphids taken directly from plants and placed on infected leaves were more difficult to study than aphids given a period of preliminary fasting; many of the former did not attempt to feed on the infected tobacco leaves and had to be rejected after 5 or 10 min. Those that did attempt to feed often made feeding punctures lasting over a minute, whereas the first feeding punctures of fasted aphids usually lasted about 30 sec. Non-fasted aphids were allowed two or three feeding punctures on the infected leaf, and at least one of these usually lasted less than a minute. On the test leaves non-fasted aphids were again slow in attempting to feed, and an aphid usually took more than 1 hr. to make five to ten feeding punctures. Although most of the feeding punctures were brief, many lasted for several minutes and some for hours. For example, of twenty-five non-fasted aphids observed, seven made feeding punctures exceeding 20 min.; by contrast, none of forty previously fasted aphids made a feeding puncture of this duration. These differences may account for some of the increased efficiency of vectors caused by fasting.

Only seven out of twenty-five non-fasted aphids observed caused infections. The durations of the feeding punctures of these are shown in Table 6. The differences between the durations of the feeding punctures of infective and non-infective aphids in this experiment were not significant, but it is of interest that only one of the infective aphids made a feeding puncture which lasted over 20 min.

Table 7 shows the results from Table 6 grouped according to the duration of the feeding punctures up to and including the last infection caused by each aphid. As with fasted aphids, there is no increase in the probability of infection with feeding punctures maintained longer than 20–30 sec.

The results in Table 6 show that the probability of infection with successive feeding punctures also decreases with non-fasted aphids. Watson (1946), working with *M. persicae* and sugar-beet mosaic virus, found that fasted aphids lost infectivity more rapidly than non-fasted ones. DWV behaves similarly when the

decrease in infectivity is measured in terms of the time spent on the test plant. The non-fasted aphids, however, were slower in attempting to feed and this difference itself would extend the period of infectivity. As shown in Fig. 1, there seems to be no consistent differences in the rate at which fasted and non-fasted aphids lose infectivity when the loss is measured in terms of successive feeding punctures.

TABLE 6. *Duration in seconds of feeding punctures of non-fasted infective* Myzus persicae

	Successive feeding punctures										
Aphid	1	2	3	4	5	6	7	8	9	10	Total
1	60	**35***	—	—	—	—	—	—	—	—	1/2
2	25	30	**30**	10	—	—	—	—	—	—	1/4
3	**40**	**10**	**60**	**30**	30	—	—	—	—	—	4/5
4	**120**	20	5	5	30	30	20	—	—	—	1/7
5	**15**	20	**40**	15	30	3000	15	240	15	—	2/9
6	**25**	30	10	15	**25**	10	365	**35**	10	35	3/10
7	**20**	**20**	15	**10**	**20**	**15**	**20**	**25**	25	**10**	8/10
Total	5/7	3/7	3/6	2/6	2/5	1/4	1/4	2/3	0/3	1/2	20/47

* Figure in black indicates feeding puncture caused an infection.

TABLE 7. *Part played by the duration of the feeding puncture of non-fasted aphids in causing an infection*

	Duration of feeding puncture					
	10–20 sec.	20–30 sec.	30–40 sec.	40–60 sec.	1–7 min.	Total
Total	5/9*	7/10	4/6	3/4	1/2	20/31

* Denominator is number of feeding punctures observed and numerator is number that caused infections.

TABLE 8. *The effect on transmission of disturbing* Myzus persicae *during the test-feeding period*

	4 hr. preliminary fasting		0 hr. preliminary fasting	
	Disturbed on test leaf	Not disturbed on test leaf	Disturbed on test leaf	Not disturbed on test leaf
Aphids infective out of 40	28	30	11	9
Percentage infective	70·0	75·0	27·5	22·5
Starch-iodine lesions caused by infective aphids	99	45	15	12
Lesions per infective aphid	3·5	1·5	1·4	1·3

The following experiment was made to test the effect on the transmission of DWV of disturbing aphids during the test-feeding period. The preliminary fasting periods were 0 and 4 hr., and each aphid was allowed to make one or two infection-feeding punctures before being placed on a tobacco leaf. During the test-feeding, half the fasted and half the non-fasted aphids were moved at 10 min. intervals.

Table 8 gives the results of eight such experiments and shows that 72 % of the fasted and 25 % of the non-fasted aphids were infective. The number of lesions produced per infective aphid, however, was approximately the same for all the treatments, except that the previously fasted aphids disturbed during the test-feeding period caused more than twice as many as any of the others. At first sight these results suggest that fasted aphids acquire more virus during a short infection-feeding period and can thus cause more infections than non-fasted aphids. This interpretation would not be justified unless aphids in both groups responded the

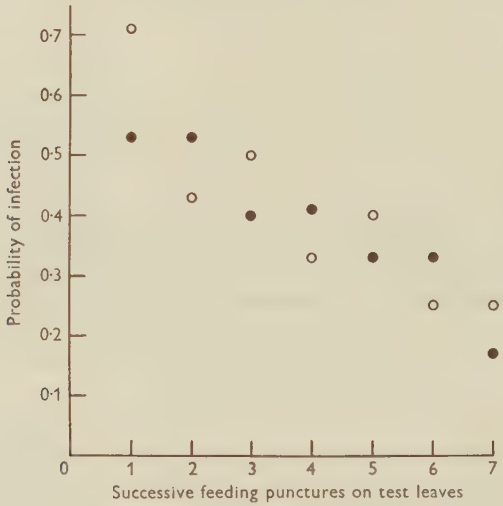

Fig. 1. Decrease in the probability of successive feeding punctures causing infections when *Myzus persicae* transmits datura wilt virus. ○ = fasted; ● = non-fasted aphids.

same way to being disturbed, and differences in the behaviour of fasted and non-fasted aphids were observable. The most noticeable was that fasted aphids attempted to feed more readily than did the non-fasted ones. When disturbed and moved with a brush, fasted aphids renewed their feeding punctures almost immediately, whereas non-fasted aphids usually wandered about the leaf making no further attempts to feed. Therefore moving at 10 min. intervals increased the number of times fasted aphids attempted to feed but had less effect on the non-fasted aphids, and may even have reduced the number of feeding punctures.

Sylvester (1949*a*) found that disturbing *M. persicae* during the test-feeding period when transmitting sugar-beet yellow net virus increased the percentage of infective aphids. Similar treatment did not affect the percentage of aphids becoming infective with DWV (Table 8). This difference in results is probably caused by a difference in the virus-vector relationship of these two viruses; yellow net virus persists in *M. persicae* whereas DWV does not.

The results of these experiments show that *M. persicae* may infect a plant with DWV during 5–10 sec. feeding punctures, and there is no increase in the probability of infection occurring during a single feeding puncture when such a feeding puncture lasts over 20–30 sec. There is no reason to believe that the amount of transmissible virus carried by infective aphids varies enough to influence an aphid's infectivity. Although, during a 5 min. period on infected leaves, a much higher percentage of fasted aphids will become infective than those having no preliminary fasting period, infective aphids in both groups infect during short feeding punctures and with a similar regularity. Some of the differences between the percentages of infective aphids obtained by varying treatments may be partly caused by differences in the feeding behaviour of fasted and non-fasted aphids.

Some observations on feeding puncture of Myzus persicae *into epidermal cells*

Using a capsule similar to that described by Hamilton (1935), stylets of *M. persicae* were observed under the high power of a microscope as they passed through living epidermal cells. The capsule was made from a glass ring 1 cm. long and 0·6 cm. in diameter. One end of the ring was dipped into melted embedding wax, and a piece of leaf epidermis about 1 cm. square stretched over it; the epidermis was secured over the end of the ring by melting the wax between the glass and the epidermis with a warm scapel. The outer surface of the epidermis was placed inwards on the glass ring. Epidermis from tulip, tobacco and cabbage was used, but tulip epidermis was the easiest to prepare and handle; it was obtained by stripping the epidermis from the dorsal side of tulip laminae, and carefully washing the inner side free from the remaining mesophyll tissue. When epidermis from cabbage or tobacco was used it was prepared by a method similar to that described by Hamilton (1935).

Ten fasted aphids were confined in the capsule by placing it, open end downwards, on a glass slide. The slide could be moved about with the stage micrometer of a microscope and allowed light from the substage to pass through the chamber and on to the epidermis. When aphids found their way to the epidermis they were upside-down, the position in which they customarily feed on the lower surface of leaves. Under the low power of the microscope an aphid could be watched until it touched the epidermis with its proboscis and began the feeding puncture. Then the tip of the proboscis could be focused under higher magnification and the advancing stylets observed. A drop of water or weak sucrose solution on the epidermis prevented it from drying and served as a medium for the stylets to puncture.

About 1 min. after the proboscis touched the epidermis (if the feeding puncture lasted a minute) the stylets usually appeared through the epidermis and into the solution above. It was not always possible to determine if the stylets passed through or between the epidermal cells, but of twenty-two occasions when this was possible, ten were intercellular and twelve intracellular. Sometimes the stylets first passed intercellularly but later became intracellular. More rarely the stylets moved horizontally through the epidermal cells, sometimes passing through as many as eight

successive cells. At these times as the stylets advanced at right angles to the line of vision, they could be observed in detail.

A colourless material which rapidly sets to a gel flows from the tip of the stylets as they advance through a cell. This gelling material, the insect's saliva, covers the tip of the advancing stylets within the leaf and when these are withdrawn the salivary sheath is left in the leaf tissue.

During the feeding puncture, *M. persicae* appears to push with its head causing the proboscis to shorten like a telescope and the stylets to be forced into the leaf. The advancing stylets appear to be assisted by a drilling-like action of the mandibular stylets vibrating back and forth. Passing through the vacuole of a cell the stylets sway to and fro until they strike the narrowing walls on the opposite side; the tip of the stylets fix a point on the cell wall, saliva is ejected, the drilling-like action of the mandibles continues and soon the stylets penetrate into the next cell. To penetrate a cell wall and to pass through to the opposite side of that cell requires approximately 1 min.; on one occasion an aphid's stylets passed through eight epidermal cells in 10 min.

Sometimes the stylets remained motionless in a cell and the aphid appeared to be feeding. If the tip of the proboscis was observed during this time, a vibration caused by a pumping-like action within the proboscis could be seen; this was probably the pharyngeal pump at work. On one occasion, when this vibration continued for 3 min., the cell became plasmolysed.

More often, however, the stylets passed through the epidermis into the liquid on top of the membrane. In liquid the stylets advanced more jerkily and with vigorous swinging movements. The saliva, which was continuously ejected in front of the stylets, was moulded into a tubular sheath. Aphids fasted for 2 days sometimes continued injecting saliva and building branching salivary sheaths for 20–30 min. without stopping. Sometimes the stylets would rest half way withdrawn in the salivary sheath.

Storey (1939) observing *Cicadulina mbila* puncturing through a wax membrane into a suspension of indian ink particles in 10% sucrose, reported that the particles flowed toward the tip of the insect's stylets as if fluid was being sucked in by the leafhopper. I have tried to obtain similar evidence that *Myzus persicae* will feed in a liquid medium. *M. persicae* was observed puncturing into 1, 5, 10 and 20% sucrose solutions at pH values ranging from 4 to 9 and containing suspensions of indian ink or puff ball spores measuring 2–4 μ in diameter. Around 100 punctures which lasted from less than 2 min. to over 20 min. were observed, but not once were either indian ink or spores observed to flow towards the tip of the stylets. Sometimes particles became entangled in the gelling saliva, caught by the swaying to and fro of the stylets. When the stylets were at rest, particles were usually so close to the salivary sheath that the slightest movement of liquid ought to have been detected; nor did any of the particles appear to be repelled by the stylets. Sometimes the stylets were motionless as if feeding but the pumping-like vibration

within the proboscis, often seen when the stylets were in epidermal cells, was never observed.

Hamilton (1930, 1935) has shown that *M. persicae* takes up eosin, methylene blue and polonium, when confined in a capsule similar to the one used for my observations. It is possible that *M. persicae* sometimes feeds on fluids and that my observations were too limited to detect this. However, another explanation of this apparent discrepancy is that the indicators used by Hamilton diffused into the epidermis used as a membrane and were taken up by aphids feeding in the epidermal cells.

If aphids were taken from their host plant and placed directly in the feeding capsule, few would attempt to feed during the first hour, during which time they became fasted aphids; usually before being placed in the capsule, aphids were fasted for several hours. As on a leaf, the first few feeding punctures of fasted aphids usually lasted less than a minute. During these short feeding punctures, the stylets penetrated only superficially into the epidermis; the stylets were barely visible under the high power of the microscope and no saliva could be seen being ejected. Usually the stylets were withdrawn in less than a minute or they proceeded to pass through the epidermis and required from 1 to 2 min. to do so; sometimes these feeding punctures with the stylets barely in an epidermal cell lasted several minutes and the pumping-like vibration within the proboscis suggested the aphids were feeding.

There is no reason to suspect that these brief feeding punctures differed from those lasting less than a minute in whole leaves, during which *M. persicae* acquires or infects with DWV. Thus, when *M. persicae* acquires or infects a plant with DWV, its stylets are probably only superficially within the epidermal cells and during these short feeding punctures little or no saliva is injected into the plant. If, as is generally thought, such viruses are injected into a plant with the insect's saliva it seems strange that the probability of infection does not increase during long feeding punctures when presumably more saliva is ejected.

Further evidence that virus is acquired from the epidermal cells was obtained in the following experiments. The mesophyll tissue of tobacco leaves infected with DWV was destroyed by pressing the leaves between the thumb and forefinger, care being taken not to break the epidermis. Most of the mesophyll cells were broken leaving the upper and lower epidermis enclosing the crushed cells. A disk of the crushed leaf was placed in a glass ring 1·5 cm. in diameter and groups of five to ten aphids confined in the ring for about 5 min. As a control, similar groups of aphids were confined in another ring with a disk of infected leaf normally used as a source of virus. In six experiments, 173 aphids placed in the glass rings on crushed infected leaf disks caused eighty-seven lesions, compared with fifty-one lesions caused by 102 aphids placed on uncrushed leaf. Thus destroying the mesophyll did not affect acquisition of DWV in *M. persicae*.

In further experiments, pieces of epidermis from infected leaves were stretched over moist blotting-paper and tested as infection sources. Pieces of epidermis were

obtained as strips from the mid veins of infected leaves or by scraping the lower epidermis and mesophyll from the upper epidermis using a smooth plastic pot label; each strip of epidermis was carefully washed free from all mesophyll. On five occasions aphids failed to become infective from these pieces of epidermis. In three later experiments, however, aphids became infective when allowed to make feeding punctures into strips of epidermis which were removed from the mid veins of infected leaves with greater care and kept moist throughout the experiment. In these experiments those aphids making feeding punctures into the epidermis during the first hour were tested on one plant and those during succeeding hours on different plants. Only aphids tested during the first hour became infective; of these, thirty aphids caused twenty-eight lesions on *Nicotiana rustica* compared with twenty-five lesions caused by twenty aphids each allowed to make one feeding puncture into an infected leaf. These results show that *Myzus persicae* can acquire DWV from the epidermal cells and it seems probable that they usually do so.

Contamination of stylets of Myzus persicae *with viruses*

When settled on a plant, *M. persicae* usually feeds continuously for hours at a time without withdrawing its stylets. If disturbed, an aphid jerks its head several times while apparently pulling its stylets from the leaf. After being disturbed the stylets can be seen, with the aid of a hand-lens, extending beyond the tip of the proboscis. When pulled from a leaf quickly, the stylets protrude from the proboscis, because the proboscis shortens like a telescope when the stylets are forced into the leaf and does not extend itself immediately the stylets are withdrawn. This is not normal. When an aphid voluntarily withdraws its stylets, it does so gradually and the proboscis slowly lengthens to cover the retracting stylets. When an aphid is disturbed it usually takes the proboscis about a minute to extend to its full length and cover the stylets which normally rest in the labial groove. The stylets were observed under the microscope by disturbing continuously feeding aphids and dropping them into hot alcohol. The stylets of *M. persicae* consist of two pointed mandibles, and a pair of maxillae which appear to be permanently fused from the distal end to the head. The mandibles lie on either side of the maxillae and appear to be capable of independent movement. The fused maxillae form two ducts: a dorsal larger one through which the insect's food passes, and a much smaller ventral duct through which saliva is ejected.

Sukhov (1944) has suggested that *M. persicae* fails to transmit tobacco mosaic virus because this virus does not pass through the proteinaceous sheath which surrounds the insects' stylets while feeding. To learn if aphids would transmit either tobacco mosaic virus or DWV when the stylets were contaminated with them, aphids were disturbed during continuous feeding and the protruding stylets dipped into solutions of these viruses. In three experiments, the stylets of over 100 aphids were dipped into drops of sap from tobacco plants infected with DWV. None caused infections when later fed on *Nicotiana tabacum* or *N. rustica.* In three

other experiments, over 100 aphids treated similarly with a concentrated solution of purified tobacco mosaic virus failed to infect *N. tabacum* or *N. glutinosa*. Drops of the virus preparations used in all six experiments were rubbed on leaves of *N. glutinosa* or *N. rustica* and caused many lesions. This suggests that DWV is not carried externally on the stylets when normally transmitted by *Myzus persicae*.

The effect of periods of darkness on the susceptibility of Nicotiana rustica *to infection by datura wilt virus*

Bawden & Roberts (1947, 1948) have shown that periods of darkness or reduced illumination previous to mechanical inoculation increased the susceptibility of French bean and tobacco to tobacco necrosis virus; of *N. glutinosa* to tobacco mosaic and tomato bushy stunt viruses; and of tobacco to tomato aucuba mosaic virus. Experiments were made to see whether this also applied to DWV when transmitted to *N. rustica* by rubbing and by aphids.

Plants subjected to periods of darkness previous to inoculation were kept in a cage similar to the one used by Bawden & Roberts (1948), which excluded all direct light. The experiments were made during summer, and plants which had been in the cage for either 2 or 4 days were compared with plants that had been maintained under normal glasshouse conditions of illumination. In a preliminary experiment the treatments were 0, 2 and 4 days in darkness. After an hour's preliminary fasting, aphids were placed on an infected leaf for about 5 min. and then transferred to the test plants. Thirty aphids were placed on each of six plants treated in different ways; the leaves of four plants in each treatment were also rubbed with infective tobacco sap. The aphids caused totals of 184, 188 and 176 local lesions on plants in darkness for 0, 2 and 4 days respectively, and the rubbed leaves gave an average of eight, sixteen and twenty-two lesions per leaf respectively.

Further experiments were carried out during which only one aphid was tested on each leaf of *N. rustica* so that the results of individual aphids could be obtained.

TABLE 9. *The effect of periods of darkness on the susceptibility of* Nicotiana rustica *to infection with datura wilt virus by aphids and by mechanical inoculation*

	Aphids				Mechanical inoculation			
		Days in dark				Days in dark		
Exp. no.	No. of aphids tested for each treatment	0	2	4	Inoculum	0	2	4
2	25	17/29*	16/31	16/34	Sap 1/1+celite	87†	222	229
3	20	14/31	14/34	9/22	Sap 1/5	4	10	9
					Sap 1/5+celite	12	48	45
4	15	10/24	10/23	8/24	Sap 1/1	13	30	52
					Sap 1/5+celite	16	113	108
Totals	60	41/84	40/88	33/80				

* Numerator is number of aphids infective and denominator is number of lesions caused by infective aphids.

† Lesions per half leaf.

Again the treatments were 0, 2 and 4 days in the dark, and fasted aphids were used after having one feeding puncture on an infected leaf. In each experiment at least twelve half leaves were rubbed with tobacco sap at various dilutions with and without the use of celite. Table 9 gives the results of three experiments and shows that periods of darkness previous to inoculation increased the susceptibility of *N. rustica* to infection by mechanical inoculation, but not to infection caused by aphids. A period of 2 days' darkness increased the susceptibility of *N. rustica* to mechanical inoculation as much as did a period of 4 days. The numbers of infective aphids and the numbers of lesions per infective aphid were approximately the same for all treatments.

Discussion

The response to a period of preliminary fasting and loss of infectivity within hours of leaving infected plants is a property of henbane mosaic, cucumber mosaic, potato *Y*, tobacco etch, beet mosaic, lettuce mosaic, pea mosaic, cabbage blackring and cauliflower mosaic viruses, transmitted by *Myzus persicae* and other aphids. These viruses, and undoubtedly many others, are probably transmitted by their aphid vectors in a similar manner. Several hypotheses have been advanced to explain this type of transmission. Doolittle & Walker (1928) suggested that cucumber mosaic virus was carried on the insect's proboscis: 'the minute quantity of virus thus carried being exhausted during the first feeding period'. Whether they meant that virus was carried on the tip of the aphid's proboscis or on the stylets which puncture into the leaf is not stated.

Hoggan (1933) first suggested that this type of transmission was more than a mere transfer of infective sap on the insect's mouthparts, when she failed to transmit tobacco mosaic virus by *M. persicae*. Tobacco mosaic virus was far easier to transmit by needle inoculation than cucumber mosaic virus, yet aphids failed to transmit it and readily transmitted cucumber mosaic virus. She concluded that the rapid transmission and loss of infectivity indicated that this virus was not transmitted through the salivary glands and that the stylets retain a 'minute quantity' of infective sap which is injected into the next plant on which the aphid feeds. She was unable to decide whether the sap was carried externally or within the aphid's mouthparts.

There is much evidence that viruses of this type are not transmitted as external contaminants of the mouthparts. The specificity between some vectors and the viruses they transmit, in itself, seems incompatible with the idea. Furthermore, *M. persicae* did not infect healthy plants after its stylets were contaminated externally with tobacco mosaic or DWV.

Watson (1936), working with henbane mosaic virus and *M. persicae*, showed that the entire process of transmission could be completed in a few minutes. Nevertheless, she considered that transmission was more than a mechanical transfer of infective sap on the aphid's stylets. She suggested that virus in an aphid might be inactivated by antibodies formed in the vector's blood, a suggestion that implied

that during transmission this type of virus comes in contact with the vector's blood.

Watson & Roberts (1940) showed that *M. persicae* could infect several plants with henbane mosaic, severe etch, or potato *Y* viruses, if given 5 min. feeding periods on a series of plants immediately after a short infection feeding period. Furthermore, aphids moved at 5 min. intervals remained infective longer than those moved at 20 min. intervals. They pointed out that, if virus is carried externally on the mouthparts, the more often the stylets are inserted and withdrawn from a leaf the more rapid any cleansing process ought to be; contrary to expectation, however, the aphids remained infective longer if moved at frequent intervals. Watson & Roberts (1940) classified plant viruses transmitted by insects into two main types based on the time vectors remain infective; these they called persistent and non-persistent viruses. They did not consider it necessary to postulate any difference between the mechanism of transmission of these two types, and suggested that the difference in the time for which vectors remain infective is explicable because non-persistent viruses are inactivated by substances in the aphids that do not inactivate persistent viruses. They advanced the hypothesis that such substances are produced by aphids while feeding, but are either not produced, or produced in much smaller quantities, by aphids while fasting. This hypothesis can be extended to fit most of the experimental facts, including perhaps the specificity of transmission. If such substances occur in aphids, however, there is no information on where they are produced or come in contact with the virus.

Though there is strong evidence against such viruses being carried as external contaminants on the stylets, there is little additional evidence to suggest where non-persistent viruses are carried by aphids. The time required for the transmission of non-persistent viruses by their aphid vectors has been shown to be less and less the more these viruses have been studied; recently Sylvester (1950*a*) has shown that *M. persicae* can acquire and transmit beet mosaic virus in less than a minute, and I have obtained transmissions of DWV by *M. persicae* equally as rapidly. These results seem to preclude passage through the gut wall into the blood thence to the salivary glands as is thought to happen with persistent viruses. There remains Hoggan's (1933) original suggestion that virus may be carried within the mouthparts.

From what is known about the internal structure of an aphid's stylets, there are two ducts where virus might be carried; one through which the food passes is connected with the pharynx, and the other is a much smaller ventral duct through which saliva is ejected into the plant. There is no known connexion between the two ducts and if virus is carried in one of these, it is probably taken into and ejected out of the same duct.

The results with DWV show that *M. persicae* usually acquires and transmits this virus during brief feeding punctures, when the stylets are inserted only into the epidermal cells and little or no saliva is ejected. Considering that there is no evidence that an aphid can acquire or transmit non-persistent viruses during continuous

feeding when the stylets are surrounded by a well-formed salivary sheath, the results with DWV suggest the following working hypothesis. The salivary sheath may normally act as a filter for the insect's food, and its absence during brief feeding punctures may lead to one of the ducts in the stylets becoming obstructed and so cause the aphid to clear it by forcing liquid outwards and finally to withdraw its stylets in search of a more suitable feeding site. The duct may still be obstructed when the aphid attempts to feed again, and while clearing it some material in the duct from the previous feeding puncture may pass into the cell. As long as aphids attempt to feed without building a suitable salivary sheath, they are likely to acquire and transmit virus, but when they feed normally and a salivary sheath is formed, either virus does not pass through the gelling material or, the duct being no longer obstructed, infective fluid is unlikely to be ejected into subsequent feeding sites.

This hypothesis explains many facts, such as the independence of infectivity from duration of feeding puncture. Indeed, the shorter the period on the infected leaf the fewer aphids will have commenced normal feeding, and the higher the percentage that will transmit. It also seems reasonable that a period of preliminary fasting will increase the number of aphids that will attempt to feed without a salivary sheath and thus increase the percentage of infective aphids. After becoming infective, the longer that aphids are prevented from settling down (such as being moved to a series of plants at short intervals), the longer they will remain infective. However, this hypothesis does not explain why aphids fail to transmit tobacco mosaic or potato *X* viruses, nor the specificity between some viruses and their aphid vectors. So little is yet understood about these phenomena, that any attempt to explain them would be premature.

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SOME EFFECTS OF A PLANT VIRUS ON NUCLEAR DIVISION

By JOHN CALDWELL

Department of Botany, University College, Exeter

(With Plate 6)

This paper records some observations on the effect of the virus of aspermy disease of tomato on the formation of the mega- and micro-spores of the plant. The obvious interference by the virus in the normal meiotic processes suggests that this is the cause of the non-formation of seed in infected plants. It is suggested that the interference by virus with nuclear divisions may be more widespread and of greater biological significance than is at present realized.

The problem of seed transmission of viruses has for long been of interest to virus workers and is clearly of great practical as well as of theoretical importance. In this note seed transmission implies the passage of the virus in the embryo proper and not in the testa or in tissue debris on the testa (cf. Ainsworth, 1933). It has already been shown (Caldwell, 1932) that accidental infection of the seedling from the testa through a broken hair is improbable though theoretically possible. Virus infection being characteristically systemic the presence of virus on or in the testa is highly probable and any virus which has a long survival period in dead tissue could, presumably, remain active in this region for a long time, as has been repeatedly shown for tobacco mosaic virus in tomato. Earlier work (Caldwell, 1932), subsequently confirmed, indicates that this virus is unable to enter an unbroken protoplast and that movement of the virus takes place through protoplasmic strands from cell to cell. It has been noted by various workers that the absence of virus in the seed is surprising, since the virus is generally distributed fairly widely throughout the cells of the plant, including the fruit (cf. Bawden, 1950). The purpose of the present note is to show that for one virus, at least, the absence of virus in the seed is not only explicable but to be expected and to suggest that the explanation probably applies to other viruses.

In the author's experience, over many years and with thousands of seedlings, no evidence has been found of virus in the embryo, that is, of seed transmission in (*a*) tomato and tobacco varieties or *Nicotiana* species with tobacco mosaic viruses, (*b*) broccoli with broccoli mosaic virus (cf. Caldwell & Prentice, 1942), (*c*) *Narcissus* species and varieties with narcissus stripe virus (cf. Caldwell & James, 1938; Caldwell & Prentice, 1942), (*d*) raspberry varieties with raspberry mosaic virus, (*e*) dahlia with dahlia mosaic virus, and (*f*) vegetable marrow with cucumber mosaic virus. Healthy seedlings, which grew into healthy plants were grown from seeds

obtained when infected plants were used both as pollen and as seed parents, the only exception being lettuce with lettuce mosaic virus.

Caldwell (1934) suggested that the absence of virus in the embryo is associated with the fact that at a very early stage of development there is a breakdown in the protoplasmic continuity between the cells of the nucellus, which presumably might contain virus in a diseased plant, and the embryo-sac and again between the embryo-sac cells and the embryo proper. If most viruses cannot enter an unbroken protoplast but only broken protoplasts initially and subsequently pass along the protoplasmic strands, then it follows that infected embryos can arise only from infected megaspores or perhaps occasionally from infected pollen grains. Secondary infection of the embryo is not likely despite the fact that the nucellar and ovular tissues may and probably do contain virus. Caldwell (1934) suggested that with tobacco mosaic virus the absence of seed-transmission might be associated with the fact that the development of the virus was prevented by some characteristic of the mega- or micro-spore mother cells and that, consequently, primarily infected embryos could not be developed. In this note the effect on embryo-sac and pollen-grain formation of a virus disease of the tomato found some three or four years ago (Blencowe & Caldwell, 1949) is discussed. The virus causing this disease is recoverable from a large number of varieties of the cultivated chrysanthemum. The effect of the disease in tomato and in various species of *Nicotiana* indicates that the virus prevents the formation of normal tetrads in micro- and mega-spore development.

Observations on the aspermy disease of tomato

This disease appears to be very common in chrysanthemum stocks and to be transmissible from them to the tomato. The effect on the tomato plant is to retard the development of, or actually to kill, the stem apex and to induce stunting in the plant followed by much development of axillary buds, associated with some distortion of the leaves and stems with a marked development of purple pigment and some chlorosis. The effects on the vegetative parts of the plant are unusual and characteristic. The effects on the floral parts are even more marked. Many of the flower buds become necrotic and drop off. Those that do not, develop into flowers, often with abnormal petals and sepals, but primarily abnormal in that the fruit produced is seedless or at most contains few seeds.

A detailed examination of the stamens of the diseased plants shows that usually no normal pollen grains are formed and there is never any appreciable amount of normal pollen. In a series of observations on healthy and diseased plants of the variety Stonor's Money-maker counts were made on the number of normal pollen grains recognizable in pollen shaken out of mature anthers. The percentage of apparently normal grains varied from 49 to 89 % in normal plants and from 0·0 to 0·8 % in diseased plants.

The malformation of the pollen grains appears to be associated with the failure of the microspore-mother-cell nucleus to go through the normal process of meiosis

leading to the formation of a tetrad of microspores. The presence of virus in the microspore-mother-cell results in a complete interference with the normal stages of meiosis and in some instances a number of cells are formed with a variable number of chromosomes. None of these cells forms a normal pollen grain and even when tetrads are formed they abort before maturity. As a consequence, the pollen sacs contain a variable number of shrivelled and distorted cells demonstrably not normal pollen grains. In most of the material examined, either as aceto-carmine smears or in sections of material fixed in Carnoy for 30 sec. followed by Belling's modification of Navashin's fluid, with the usual precautions, and stained by the iron alum-haematoxylin method, it has been found that at pachytene the chromosomes aggregate themselves into an irregular mass which stains darkly but has little recognizable structure. Apparently in association with this condition excessive vacuolation occurs in the pollen-mother-cells. When this has occurred it is followed by a disintegration of the cell and the occurrence of a mass of dead cells in the pollen sac. This 'collapse' of the chromosomes in the nucleus at the onset of meiosis has been seen in all the cells of a single pollen sac in some cases and has been noted in all the many stamens of diseased material examined. It has not been observed in the stamens of healthy material grown and prepared under exactly similar conditions at the same time and so cannot be dismissed as an artifact due to imperfect fixation. Occasionally a few apparently normal pollen grains are formed and the significance of these is noted below. Pl. 6, fig. 1 *a*, *b*, shows the appearance of the diseased microspore-mother-cells at the first meiotic prophase compared with normal cells at the same stage. Both sets of material were obtained from anthers of the variety of tomato Stonor's Money-maker.

A similar set of observations was made on the developing ovules in the fruit of the same variety: again both healthy and infected with the aspermy virus. The meiotic processes were so disorganized by infection that no normal reduction division took place, the nucleus of the megaspore-mother-cell being unable to undergo a regular meiosis, with the result that no megaspore or embryo-sac was formed and the cells of the nucellus were found to surround a few disorganized cells or a mass of protoplasm and nuclear material which rapidly became necrotic and collapsed. Pl. 6, fig. 2 *a*, *b*, shows examples of the preparations obtained. This same aggregation of the chromosomes at pachytene accompanied by precocious nucleolar disintegration was noted in the megaspore-mother-cell nuclei as is described above for the pollen-mother-cells. This was repeatedly observed in diseased material but never in the many ovules of healthy material examined. Both materials were grown and prepared at the same time under identical conditions. Here again, an occasional embryo-sac (less than 1%) was found to be apparently normal.

Discussion

The observations recorded in this paper indicate that the suggestion made by the author in 1934 to account for the absence of seed transmission of tobacco mosaic virus by an embryo must be modified. The effect is not apparently one of the spore-mother-cell on the virus but of the virus on the spore-mother-cell. It was suggested then, on circumstantial evidence, that since secondary infection of the embryo was unlikely as there was no protoplasmic connexion between it and the tissue of the parent plant, primary infection—i.e. the formation of a megaspore from an infected megaspore-mother-cell must be improbable as embryo transmission of the virus under observation was not found. The fact that in most of the common mosaic diseases of tobacco and of tomato the fairly large number of seeds with normal healthy embryos suggests that in plants so infected the number of infected megaspore-mother-cells is for some reason not great. In aspermy disease the occurrence of virus in the megaspore- and microspore-mother-cells is much more regular than in the former cases. The occurrence of an occasional viable seed and of a few normal pollen grains in diseased plants is accounted for by the suggestion that a few megaspore- and microspore-mother-cells do not contain virus and will therefore divide and behave normally. It is now clearly established by numerous workers that the amounts of virus in plants affected by a virus of systemic occurrence is by no means constant throughout the whole plant but varies considerably from place to place in the leaf and in other tissues. Under these circumstances it would seem reasonable to assume a corresponding variation in the amounts of virus in single cells or groups of cells some of which may have no virus in them.

It is interesting to note that in 1933 Kostoff called attention to the fact that in some mosaic-diseased tobacco varieties and related *Nicotiana* species non-functional pollen and/or non-functional ovules were formed. 'In the most severely attacked buds single or several pollen-mother-cells occasionally degenerated during diakinesis.' He went so far as to suggest 'The abnormalities in the reduction division of the tobacco plants infected with the mosaic are, no doubt, connected with the abnormal processes that are caused by the virus' (Kostoff, 1933).

It would appear that the implications of Kostoff's observations on the effect of virus on meiosis have been overlooked by many virus workers, presumably because his interest was in the nuclear effects from a strictly cytological point of view and he was only secondarily interested in the virus aspect not even clearly indicating the 'mosaics' with which he was concerned. At the same time, that the effect of the virus on the meiotic processes is of far-reaching importance, as this note tries to show, is implicit in any consideration of the possibility of the transmission of virus in the embryo. There are many other virus diseases and hosts which have now to be re-examined in the light of the conclusions which have been obtained with the aspermy virus in tomato and tobacco. For example, transmission of virus

of lettuce mosaic in the seed of lettuce in a small percentage of seeds may indicate (*a*) that the effect of the virus or lettuce is different from that of aspermy virus on tomato, or (*b*) that the infected embryos are not formed by normal fusion of gametes produced from infected spore-mother-cells but by some parthenogenetic means not involving meiosis. These and other aspects of the problem are now under investigation in this laboratory.

In the light of the interest in virus diseases of plants and animals and of the recent work on the nature of the virus and its association with nucleo-proteins, the effects of the virus on nuclear divisions both in meiosis and in mitosis would appear to be of great biological interest. Apart from the question of the production or non-production of infected embryos, the disturbance of normal nuclear divisions may be associated with the formation of abnormal cells as described in various virus diseases, e.g. narcissus stripe, and may be the cause and not the result of the necrosis which is so commonly found in many virus diseases.

Grateful acknowledgement is made by the author of the co-operation of his departmental colleagues, especially Miss E. Kissick and Dr J. Wilkinson in the preparation of the material and photographs in this note.

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EXPLANATION OF PLATE 6

Fig. 1. First meiotic prophase in microspore-mother-cell of (*a*) diseased tomato variety Stonor's Money-maker, showing collapse at pachytene accompanied by precocious nucleolar disintegration, and (*b*) healthy control. × 1000.

Fig. 2. First meiotic prophase in megaspore-mother-cell of (*a*) diseased material of the same variety, showing commencement of pachytene collapse and lobing of nucleolus prior to disintegration, (*b*) healthy control. × 1000.

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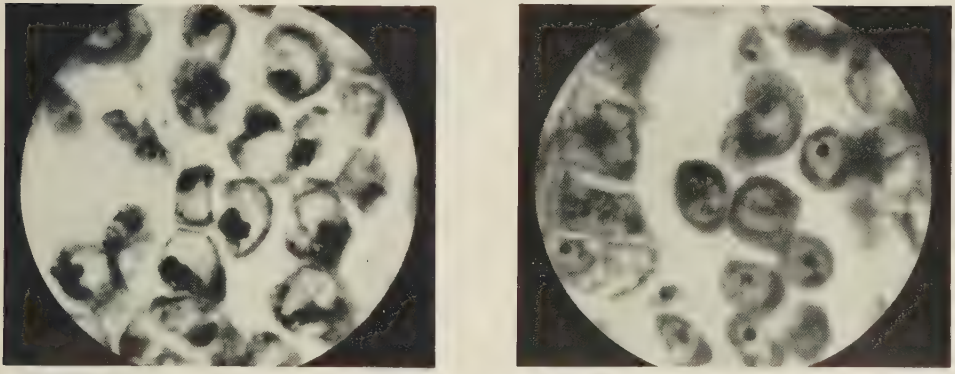
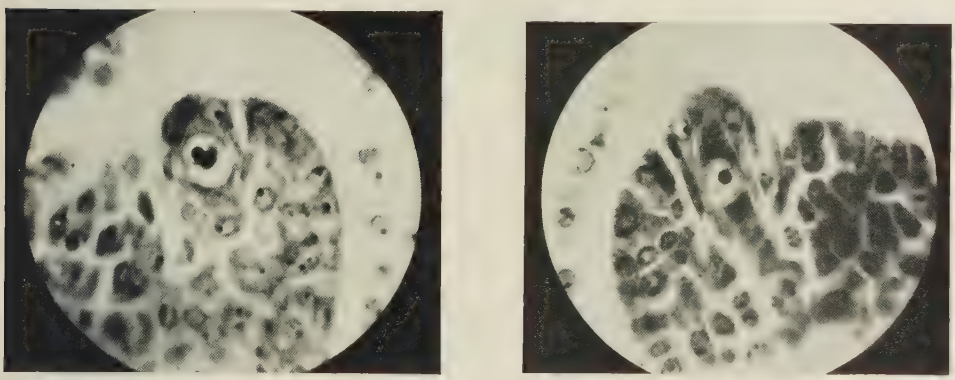


(*a*) (*b*)

Fig. 1.



(*a*) (*b*)

Fig. 2.

STUDIES OF THE CLOVE TREE

IV. NATURAL GRAFTING AND ITS BEARING ON SUDDEN-DEATH DISEASE

By F. M. L. SHEFFIELD, D.Sc.*

Clove Research Scheme, Zanzibar

(With Plates 7 and 8)

Experimental grafting between cloves is very difficult with shoots, and so far has proved impossible with roots. Use has therefore been made of naturally occurring grafts in the study of sudden-death disease. Volunteer seedlings often grow up closely adpressed to old trees. If the old tree dies from sudden-death disease, the pole† usually survives, but occasionally it dies almost simultaneously with the old tree. The poles discussed invariably had an independent root system, but those which died were found to have their roots grafted to those of the old tree whilst poles which survived, although closely adpressed to the old tree, had no organic connexion with it. These observations cannot be reconciled with any but a pathogenic hypothesis as to the nature of the sudden-death disease.

Introduction

In previous papers of this series (Nutman, 1950; Nutman & Sheffield, 1949; Sheffield, 1950) it has been deduced that sudden-death disease of cloves is caused by a pathogen but no conclusive proof has been offered. Microscopic examination enabled all types of pathogen except a virus or a fungus to be eliminated (Sheffield, 1950), and physiological studies led Nutman (1950) to conclude that the causal agent must be either a toxin-producing fungus or a virus. If sudden-death disease is due to a fungus, the fact should be susceptible to direct proof, but the intractability of the only known host plant renders application of many of the recognized virus techniques impossible. The presence of a large excess of protein precipitant (tannin) in expressed sap precludes the use of serological methods and makes it extremely unlikely that a virus could ever be mechanically transmitted from the clove (Sheffield, unpublished). Attempts by several members of the Clove Research Team to use insects to transmit the disease have so far failed. Transmission of a disease by grafting is usually regarded, in the absence of a visible pathogen, as final proof of the presence of a virus. May (1949) worked out techniques for grafting clove shoots and leaves to clove seedlings. However, the healthy seedlings to which he grafted scions from diseased trees still remain healthy after varying periods up to $2\frac{1}{2}$ years. This suggests that, if the disease is caused by a virus, it must, like

* Seconded from the East African Agricultural and Forestry Research Organization.

† 'Pole' is the term usually used to describe a clove sapling.

phony disease of peach (Hutchins, 1933), be largely confined to the roots. All attempts to graft roots together experimentally have failed.

Root grafting does occur in the field. When the old plantations were established, two to four seedlings were usually planted very close together, and all that survived were allowed to remain so that, when the trees reached maturity, they were very closely pressed together at the base. In such cases grafting is frequent. In ill-kept plantations, self-sown seedlings often grow up closely adpressed to old trees. If the old tree dies from sudden-death disease the sapling is usually unaffected, but occasionally it dies almost simultaneously with the old tree. This paper is concerned with observations made on poles closely adpressed to the old diseased trees.

Material and methods

All regions of active sudden-death disease in Zanzibar Island were searched and the positions of recently dead or 'suspect'* trees, with small poles closely adpressed to their bases, were recorded. A similar search of the southern part of Pemba was organized by Mr I. G. C. Squire of the Department of Agriculture, to whom my best thanks are due.

If both tree and pole were dead, a detailed examination of them was made at the first opportunity. If the pole only was alive, it was left until at least 6 months had elapsed since the death of the old tree. If neither were dead, the tree was visited at intervals, until either both were dead, or if the pole remained alive, until 6 months had elapsed after the death of the old tree. With one exception, which can probably be discounted, the poles that died did so either simultaneously with the old tree or slightly later.

Amongst the first trees felled were a few where, after felling, the pole was thought to have died from causes other than sudden-death disease. Later, poles were very carefully examined before felling and if any signs of ring-barking, termites, borers, etc., were found, they were discarded. Trees which had obviously been affected by die-back disease were not used, nor were any which had possibly died as a result of changing water table (Briant, 1946). It was not, of course, possible to guard against infection carried by insects, but to reduce this possibility to a minimum, a dead pole was discarded if its leaves were intermingled with those of the big tree. Unless a pole was branched, this usually corresponded to a diameter of not more than $2\frac{1}{2}$ in. at soil level. Some poles had, however, up to six branches arising near the base. Their habit was then quite different from that of the unbranched pole which, growing in the shade of the tree, tends to be very rapidly drawn up. The smallest dead pole found was $\frac{3}{4}$ in. diameter at soil level, but usually they were more than 1 in. The conditions required to be fulfilled by the live poles were slightly different

* There is no known symptom diagnostic of the disease (Nutman & Sheffield, 1949). 'Suspect' implies that an experienced observer would expect the tree to die within some months but cannot definitely state that it will.

from those of the dead poles. The minimum size was set by that of the smallest dead pole found, but no upper limit was placed on size. Their branches were often intermingled with those of the old tree, giving them maximum opportunity to become infected by insects were the disease transmitted in this way. Their frequently greater size and age also gave them greater opportunity to have become grafted or to have become infected by a root pathogen.

To examine the relation between the pole and the tree, the roots were first exposed on the side where the pole was situated. These were carefully examined for apparent unions between those of the pole and old tree. If any were found, they were sawn across to discover whether there was a true graft. In some cases roots were so closely pressed together that they were greatly distorted, or one might even be embedded in the other and remain adherent when sectioned even if there were no union between the cells. A virus will not be transmitted unless the phloems of diseased and healthy plants are fused and a phloem union is always accompanied by a fusion of at least a small portion of the xylems of the two plants. If no such graft unions were found in the lateral roots these were cut off and an attempt made to lift out the pole. If the pole were alive, it was almost invariably possible, by careful excavation and cutting of those lateral roots which examination had shown to be ungrafted, to lift the pole from the soil without felling the old tree. If this were not possible, and it seldom was if the pole were dead, the remainder of the roots were exposed and cut and the tree was pulled from the soil. The bole was cut off and washed and cut across at any levels where it appeared that grafts might be found.

Results

Old multiple trees

Where a number of seedlings have been planted near together and have become closely adpressed as they grew, if one is attacked by sudden-death disease, all ultimately succumb. It has often been noticed that the components of such multiple trees may not all die quite simultaneously. Usually the time lag is only of 1 or 2 weeks but occasionally it is longer. In a block of trees where dates of death were recorded at first weekly, but later monthly, and which contained 132 such compound trees, the components of eighty-one died at intervals too short to be recorded separately; twelve at intervals of up to 6 weeks, and in one case there was a longer lapse. This tree had three trunks, two of which were dead on 6 July 1949, and the third not until 12 October. Examination showed extensive grafting between the tap roots of two trees about 6 in. below the collar. Although the roots of the third tree were closely intermingled with those of the two grafted trees, there were no unions between them.

During the study of the relation between poles and old trees, the boles of many compound trees were sectioned. Almost invariably grafts were found between the roots (Pl. 8, fig. 5). Often, but not invariably, these unions were between the tap

roots. All such unions were below the collar, none ever being found between stems. As no records existed of the dates of death of the component trees, little could be deduced from such natural grafting.

Old trees with closely adpressed poles

Detailed descriptions of the trees examined have been deposited with The Librarian, British Museum (Natural History), and the results will be only briefly summarized here.

Both old tree and pole dead

These (Pl. 7, figs. 1 and 3) were only rarely found, but in almost every case the dead pole was found to be grafted to the old dead tree. The unions were almost invariably below the collar and might be between the two tap roots (Pl. 8, figs. 6 and 10), between the tap root of the pole and a lateral from the old tree (Pl. 8, fig. 12) or between two lateral roots (Pl. 8, fig. 9). Each type seemed to occur with about equal frequency. In some cases there was more than one union between the two trees. In one instance the tap roots of two poles were grafted together (Pl. 8, fig. 8) and two laterals from one of them were grafted to two laterals from the old tree (Pl. 8, fig. 10). One pole in this case was connected to the old tree only through the medium of the other pole. It was therefore a naturally produced equivalent of the 'chain' grafting of peaches (Hutchins, 1933).

Sometimes the tap root of the pole becomes embedded in an indentation of that of the old tree and, although often more than half the perimeter of the former is in intimate contact with the tissue of the old tree, the union seldom occurs over the whole area in contact (Pl. 8, fig. 7). More usually, the actual grafts are very small indeed and may be as little as $\frac{1}{2}$ in. in a vertical, and $\frac{1}{8}$ in. in a horizontal direction.

The tissues never seem to become grafted together, unless subjected to very great pressure. In tap roots found grafted together, friction and pressure had obviously occurred as the two roots grew in thickness. Two laterals or the tap root of the pole and a lateral from the old tree, united only if very tightly held between other larger roots (Pl. 8, fig. 11). As each component of such a group of interwoven roots grows in thickness, it becomes greatly distorted and is often crushed to less than a quarter of its normal diameter. Even in these conditions grafting is rare and, when it does occur, is never over the whole surface in contact and seems always to be between the two inner, and usually smaller, components of the group only. Even when so crushed, union seems never to occur between tissues above the collar. A pole was occasionally found much crushed between the trunks of an old multiple tree, but no graft was ever found. These observations help to explain the extreme difficulty experienced in experimental grafting of the clove. It has been achieved with young shoots or leaves as scions (May, 1949), but the percentage of success was very low and root grafting has so far proved impossible experimentally. The rarity with which it occurs in nature, except in the case of closely adpressed

old trees, is shown by the vastly greater number of living poles than dead ones, seen adjacent to trees which had died from sudden-death disease. (It will later be shown that there is a definite association between grafting and the death of the pole.)

In one case unions were found between the roots of the pole and the trunk of the old tree. This was when a seedling had germinated in the crutch of an old multiple tree and its roots had penetrated into the trunk of one component of the group. The whole group died simultaneously, and examination revealed that the pole had no independent root system and was semi-parasitic on the old tree.

To summarize the results obtained, fourteen cases can be regarded as showing good graft unions between a dead pole and a dead tree. Two others, although showing good graft unions, should probably be disregarded, as it was possible that the poles died from causes other than sudden-death disease as both had a root rot. In three cases, no tissue union was found between the dead pole and the old tree. In each, part of the top of the pole had been chopped off and in one of these cases the pole appeared to have died before the old tree. In all three the roots were completely rotten and it seemed probable that injury had rendered the plants liable to fungal attack.

Old tree dead but pole living

These (Pl. 7, figs. 2 and 4) were quite common and so a random selection was made of thirty-four live poles closely adpressed to trees dead for 6 months or more and whose diameter, at the base, was $\frac{3}{4}$ in. or more. A few were from trees which also had a closely adpressed dead pole. The roots of each live pole were very carefully exposed, and they were invariably found to be much entangled with those of the old tree. Often, the pole was so closely adpressed to the collar of the old tree that it had become almost embedded in it (Pl. 7, fig. 4). Also the tap root or laterals of the pole were often so crushed between those of the old tree that they became greatly flattened and it seemed impossible for grafting not to have occurred. Roots were carefully excavated and, where necessary, were cut after making certain that there were no grafts towards their distal ends. It was then almost always possible to lift the pole from the soil. Occasionally the roots of the pole were so entangled with those of the old tree that the latter had to be pulled from the ground, but in no case was a graft found between a healthy pole and a dead tree.

Discussion

To test whether 'grafting' and 'death of the pole' were independent, the χ^2 test was applied to the results summarized above. Those grafted poles which might have died from causes other than sudden-death disease were omitted from the calculations as also was the pole which had no independent root system. Those dead poles which were not grafted were included, although there was evidence that each of these deaths was due to a cause other than sudden-death disease.

These omissions and exceptions introduce a bias towards non-significance but, nevertheless, the value of χ^2 is 38·6. Since the value of χ^2 corresponding to a probability of only 1% is 6·635, it will be seen that the probability of a chance association is extremely low. Therefore the association between grafting and the death of the pole can be regarded as very highly significant.

It cannot be argued that the poles died only when grafted because they were dependent on the old tree for water. Great care was taken to observe that each dead pole had an independent root system which was adequate and normal. Furthermore, the organic connexion between the pole and the old tree was often very slight. The only possible explanation of these deaths is transmission to the poles through the graft unions. Only a toxin or a pathogen could be transmitted in this way. Unless produced by a fungus, no toxic substance could enter the large tree without also invading any closely adpressed pole. That poles grafted to diseased trees die, whilst similarly situated but ungrafted poles do not, therefore affords overwhelming evidence of the pathogenic nature of sudden-death disease. There was already much circumstantial evidence to suggest this, and the various forms of pathogen which might be concerned have been discussed (Nutman & Sheffield, 1949; Sheffield, 1950). Sheffield (1950) was able to eliminate all possibilities except a virus or a fungus. If no visible pathogen is present, transmission of a disease by grafting is usually regarded as proof that a virus is the cause. However, the presence of fungal hyphae in the tissues of diseased clove trees has often been described (Campbell, 1940; Sheffield, 1950; Welsford, 1922), and Nutman and Roberts have recently found a specific fungus to be almost invariably present in trees which have died from sudden-death disease (unpublished). They also found that this fungus causes a yellow stain to develop in such trees. This stain was found in all the dead poles and dead trees examined, but in some it was very slight and in others very extensive. The amount of staining present seemed to vary directly with the time that had elapsed since death occurred.

The fact that seedlings grafted with scions from infected trees, by W. B. May, remain healthy after periods of up to $2\frac{1}{2}$ years appears to be against a virus hypothesis. The possibility remains that a virus is present but is confined in the roots, for he was unable to graft these experimentally. Alternatively, a virus might at some seasons be present in the tops and be inactivated at other seasons. This could occur without a virus being transmitted, although experimental graftings of seedlings were done at all seasons, for the union takes many weeks to form and a virus could be inactivated in the scion during this period.

The observations described here offer little evidence on the question whether it is a virus or a fungus that causes sudden-death disease. Poles are unaffected unless organically connected to other diseased trees. This might suggest that a fungus is the cause for, in some cases, young trees may be relatively immune to specific fungal pathogens. It is possible that this is the case with sudden-death disease and that young trees will succumb if subjected to direct inoculation. It might, however,

equally well be argued that an insect-transmitted virus is the cause and that the leaves of young trees are not intermingled with those of old trees and therefore are less likely to become infested with infective insects.

There is often, but not invariably, a slight time lag between the death of the old tree and that of the pole. (The pole appears never to die first.) This too could occur if either a virus or a fungus were the cause of death. Either would probably have to travel some distance from the point of entry into the old tree before reaching the graft and passing into the pole.

The invariable death of grafted poles, however small the tissue union, seems to be evidence in favour of a virus being the pathogen, for it might be expected that a virus would be more widespread in the tissues of the host and more likely to reach a very small union than would a fungus.

A further possibility remains. Perhaps sudden-death disease is caused by a virus which, at the same time, renders the host more susceptible to attack by particular fungi.

Experimental transmission of the disease by some method, other than grafting, must be accomplished before it will be possible to identify the type of pathogen concerned.

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EXPLANATION OF PLATES 7 AND 8

Plate 7

Fig. 1. The old multiple tree has been dead for 6 weeks whilst the pole to the right of it has just wilted.

Fig. 2. An old tree has been dead for more than 1 year. A pole which emerges from the ground to the front and then passes to the left and behind it, is still apparently healthy.

Fig. 3. Shows the base of a 25-year-old tree with two poles closely adpressed to its base. All have been dead 1 week. (The poles were cut off immediately prior to felling.)

Fig. 4. The base of a live pole is shown caught in the crutch between two large roots of an old tree, dead 6 months.

* Copies in the files of the Department of Agriculture, Zanzibar.

PLATE 8

Fig. 5. Section of bole of tree shown in Fig. 1, about 6 in. below the collar. It shows the tap roots of four old trees as well as that of the pole (just to right of centre at bottom of photograph). The tissues of three of the former are grafted together. The fourth is attached through a lateral root to the pole which is also united with one other old tree.

Fig. 6. Section of bole of a young tree with a closely adpressed pole below the collar. The tap root of the pole (shaded) is united with that of the old tree over a large area. The pole and tree died simultaneously.

Fig. 7. Section of bole of an old tree showing a pole deeply embedded in an indentation of the tap root. Union has occurred for about 2 in. in a horizontal plane at two points on the circumference of the pole.

Fig. 8. The tap roots of two poles adjacent to a very old tree were grafted together. The section shows a union extending about 1 in. in the horizontal plane.

Fig. 9. Section showing graft union between lateral roots from one of the poles seen in Fig. 8 and from the old tree. The roots have become greatly distorted by pressure between larger roots, but the union extends only about $\frac{3}{4}$ in. in the plane of the section.

Fig. 10. Section of the bole of tree in fig. 3, showing smallest pole found to be grafted to an old tree. The tap root in the section is $\frac{3}{4} \times 1\frac{1}{4}$ in. and the union extends for only $\frac{1}{8}$ in. in a horizontal plane.

Fig. 11. The lateral root of a pole has become embedded, due to pressure between large roots, in a lateral root from an old tree. The section shows unions of $\frac{1}{4}$ in. each at two points on the circumference of the root of the pole.

Fig. 12. Shows a graft, extending only $\frac{1}{8}$ in., between the tap root of a pole and a lateral root from an old tree. (The pole is to the left of the picture.)

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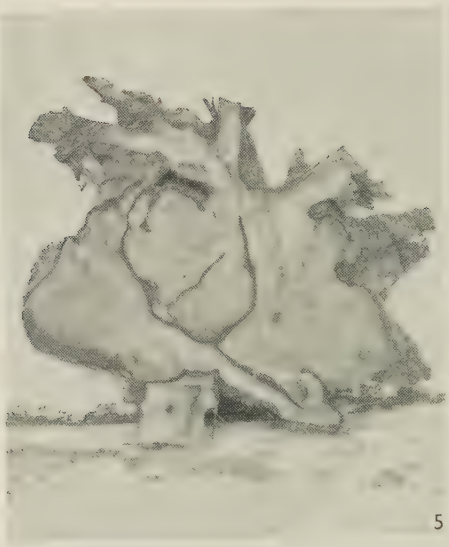
SHEFFIELD—*Studies of the clove tree*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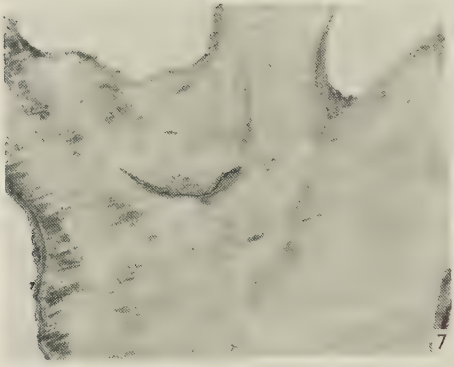
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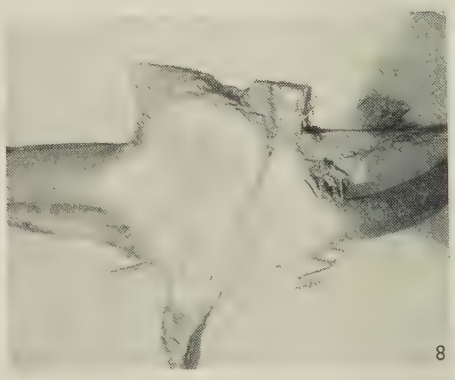
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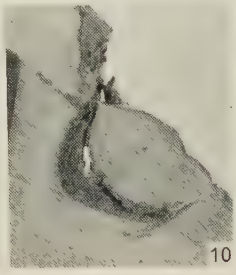
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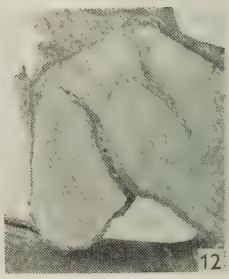
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SHEFFIELD—*Studies of the clove tree*

THE COMPETITION BETWEEN BARLEY AND CERTAIN WEEDS UNDER CONTROLLED CONDITIONS

V. COMPETITION WITH CLOVER CONSIDERED AS A WEED

By HAROLD H. MANN and T. W. BARNES

Woburn Experimental Station, Husborne Crawley, Bletchley

One of the commonest agricultural practices in Britain is the undersowing of barley and other corn crops with clover as a preparation for a clover ley after the corn crop has been reaped. The effect of the clover growing among the corn, where manuring (especially with nitrogen) is made as favourable as possible for the corn crop, is not clear. If nitrogen is deficient, it is well known that clover can furnish nitrogen for the corn, but where the supply is ample, the clover may act as a competitive crop.

When barley and clover were planted together, with abundance of water and nutrients for both, it was found that the presence of even a small number of barley plants reduced the growth of the clover by over 50%, but that an increase in the density of the barley did not increase the effect. The presence of the clover reduced the barley crop by an amount which tended to get less as the density of the barley was increased. In a sparse crop of barley, increase in clover density reduced the growth of barley very little, while that of the clover was again found to be little more than half what was obtained when this plant was grown alone.

There is no evidence of any specific effect of the roots of the one plant on the other. In the circumstances of these experiments it would seem that, far from the clover supplying nitrogen to the barley, it tends to steal some of that which would otherwise be available to the latter, when they are grown together.

Introduction

In the previous papers of the present series we have dealt with the effect of various weeds on barley and of barley on these weeds when they are grown together. In the experiments so far made the plants accompanying the barley have been known enemies of the main crop. But there are instances in which two plants, one of which is barley, are deliberately grown together, the most common of which is the undersowing of barley with clover, a practice which is almost universal when it is desired to follow the barley crop with a clover or seeds ley. The growing of clover with a gramineous crop stands on a different level from that of any of the other mixtures we have considered because the nitrogen fixed by the clover plant may benefit the barley or other crop with which it is grown. In fact, a very large number of investigations have been made in which it has been proved that, when these crops are cultivated together, and the barley or other gramineous crop is short of nitrogen, it can make use of nitrogen which has been fixed by the clover or other leguminous crop (see Virtanen, 1947; Thornton & Nicol, 1934).

The case we have studied is, however, of a rather different type. In practice, when barley or wheat is undersown with clover, the conditions are made as favourable as possible for the main crop, i.e. wheat or barley, and sufficient nitrogen as well as other manurial constituents are supplied in a readily available form to give a full yield of such main crop, so that it will not *need* to be dependent on the clover for its nitrogen supply. This is done even although there is evidence that the addition of such amounts of soluble nitrogen is rather unfavourable for the clover crop itself. Under these conditions it may be asked whether the clover behaves in any other way than as a competitive crop, in the same way as ordinary weeds such as we have described in previous papers of this series (Mann & Barnes, 1945, 1947, 1949, 1950). It was therefore decided to employ the technique used in the study of these weeds, to find the effect of an admixture of clover with a barley crop under conditions so controlled that there were ample food constituents present for full crops of both plants, and ample water, so that the only variable factor was the number of plants of each kind grown in a fixed and limited root space.

The crops were grown together in earthenware pots, 28 cm. in diameter and 25·5 cm. deep, with an upturned outlet near the bottom which enables watering to be done without danger of loss and yet secures good aeration of the soil. The bottom of each pot is covered with coarse flint gravel to a level above this outlet. Each pot is then filled with 16·3 kg. of soil taken from one of the fields at Woburn. This gives about 20 cm. depth of soil. If the volume of the gravel be included as available for the plant roots, the whole root space would be 14·2 l. per pot, and this remained constant during the experiments. Sufficient potassium phosphate and ammonium sulphate were added to supply all the phosphates and potash required by the plants and to give almost a maximum growth of the barley. Water and aeration were adequate during the growth of the plants. The only variables were, therefore, the number of plants of either barley or clover in a definite volume of soil. It will be seen that there is no attempt to make the barley depend on the clover for the nitrogen it requires. It has been established in previous experiments on similar lines that 0·75 g. nitrogen per pot would give almost a full yield of barley at any thickness of planting that we have used (see 1945) and this was the quantity employed in the present case, being equal to 0·053 g. nitrogen per litre of root space. It has also been shown that 4 plants of barley per pot will give a maximum yield of barley in the space available and that a greater thickness of sowing would not give any increase of produce.

Before attempting to measure the effect of clover on barley, it was, however, necessary to ascertain the root space needed for maximum growth of the clover. Clover was, therefore, sown alone in duplicate pots under exactly similar conditions to those provided for barley. This was done at ranges of 1 plant per pot (616 sq.cm. of surface or 14·2 l. of root space per plant) to 8 plants per pot (77 sq.cm. of surface or 1·8 l. of root space per plant). The details of growth with each thickness of sowing are shown in Table 1.

TABLE 1. *Effect of thickness of planting on yield of clover*

Clover plants per pot	Root space per plant (l.)	Area per plant (sq.cm.)	Wt. of air-dry clover above ground per pot (g.)	Wt. of air-dry clover roots per pot (g.)	Nitrogen in dry produce (%)	Root space per 1 g. root (l.)
1	14·2	616	59·1(?)	12·7	3·22	1·12
2	7·1	308	94·1	14·6	2·76	0·97
4	3·5	154	121·0	18·5	2·75	0·77
6	2·4	103	131·5	21·9	2·50	0·65
8	1·8	77	127·2	20·4	2·90	0·70

The maximum growth above ground is reached with 6 plants per pot. It appears, however, that while the roots from 1 and 2 plants per pot are not quite able, at any rate in the first year, to fill the space provided for them, a larger number is able to do so more or less completely. The way in which the clover occupies the root space available, i.e. the actual volume occupied by 1 g. of roots when the maximum growth is obtained, is far more than with the two twitch grasses previously studied, but considerably less than with barley. The suggestion made in our previous paper (Mann & Barnes, 1950) that the competitive power of a plant can be in some degree measured by the amount of root growth relative to that above ground, would suggest that clover should be a strong competitor with barley, and the reduction of growth of barley in a mixed culture (apart altogether from any question of nitrogen supply) would be greater than the effect of barley on the clover itself. It is shown later that this is not so, and that with clover certain new factors enter into the question. The relationship between the weight of roots and the weight of growth above ground for a series of plants that we have studied is shown in Table 2 as a mean for all thicknesses of planting.

TABLE 2. *Comparisons of root growth and nitrogen content in barley, clover and three weed plants*

Name of plant	Proportion of root growth to total weight (%)	Proportion of total nitrogen in roots (%)
Spurrey (1943)	10·2	7·9
Mayweed (1943)	10·1	11·4
Chickweed (1946)	16·5	14·1
Barley (1946)	11·5	13·8
Clover (1948)	14·2	14·4

One of the chief interests of this table lies in the proportion of the plant foods supplied which is fixed in the roots and so withdrawn from the above-ground organs. Clover stands apart from any of the other plants, and even when a substantial amount of nitrogen is added in the form of ammonium sulphate, the total quantity of nitrogen in clover, both below and above ground, is greater than in any of the other plants studied. The proportion of that in the roots to that above ground is almost identical with that in chickweed or barley.

COMPETITION BETWEEN BARLEY AND CLOVER

When barley and clover are grown in association, with sufficient nitrogenous fertilizer to secure almost a full crop of barley, competition arises between the plants both for space and apparently for nitrogen. Table 3 shows the yields obtained where the density of the barley was varied from a sparse to a thick crop, while the density of the clover was kept constant at 6 plants per pot. This density of the clover plants is slightly greater than is necessary to give a full crop if no other plant is present. It is therefore clear that the barley is growing in a space which would be already fully occupied by clover if the latter were grown alone.

TABLE 3. *Yield of barley and clover, with varying density of barley*

No. of plants per pot		Maximum no. of barley shoots per pot	Yield of barley, g. per pot		Yield of clover, g. per pot (air-dry)
Barley	Clover		Grain	Total	
0	6	—	—	—	131·5
1	6	27	30·9	75·9	62·4
1	0	28	38·7	101·2	—
2	6	35	34·2	83·3	63·9
2	0	35	41·0	102·0	—
4	6	47	40·7	96·7	47·6
4	0	42	43·5	103·8	—
6	6	49	38·5	92·1	52·5
6	0	41	44·7	107·5	—
8	6	54	39·5	94·8	47·8
8	0	47	42·7	100·0	—

The only sign in the above figures of any benefit to the barley from the presence of clover is in the number of shoots (tillers) produced by the barley. In no case is the number less where the barley is mixed with clover, and with the greater density of the barley plants the number of such tillers is greater when the clover is present. Table 4 indicates that the yield of ripe barley consistently shows a decrease when the clover is present, and that there is a fall in the clover yield due to the presence even of a small number of barley plants.

TABLE 4. *Percentage reduction in barley yield owing to presence of clover and of clover owing to presence of barley*

No. of plants per pot		Percentage reduction in yield		
		Barley		Clover, total produce
Barley	Clover	Grain	Total produce	
1	6	20·2	25·0	52·5
2	6	16·6	18·3	51·4
4	6	6·4	6·8	63·8
6	6	13·9	14·4	60·1
8	6	7·5	5·2	63·7

It is clear that at all levels the effect of even a small addition of barley is far greater on the clover than is the presence of a full crop of clover on a large and perhaps excessive sowing of barley. But, on the other hand, an increase in the number of barley plants per pot appears to make comparatively little difference to the clover. It is the presence of *any* barley which seems to affect the growth of clover and an increase in the amount of barley has only a minor further effect. There is little evidence that a thick seeding of barley will smother the clover, contrary to what we have found with spurrey and mayweed and, to a less extent, with chickweed. With all plants grown with barley the presence of the second plant has caused a reduction in the barley crop, and the barley has reduced the luxuriance of the weed. But the effect is very different with different plants, as is shown in Table 5.

TABLE 5. *Comparison of clover with other plants as competitors with barley*

No. of plants per pot		Percentage reduction in total produce							
		Clover		Chickweed		Spurrey		Mayweed	
Barley	Weed	Barley	Clover	Barley	Weed	Barley	Weed	Barley	Weed
1	6	25	53	90	4	36	85	61	70
2	6	18	51	87	12	33	83	42	76
4	6	7	64	78	17	10	93	35	79
6	6	14	60	82	4	5	94	22	85
8	6	5	64	62	32	Nil	94	20	87

With spurrey and clover the reduction, at any density of barley plants, is small: with mayweed it is greater: and a maximum is reached with chickweed, where, unless the barley crop is very thick, it is almost wiped out by the dense growth of the weed. On the other hand, a thick crop of barley is almost able to suppress spurrey and mayweed, but is itself very much reduced by chickweed, while clover occupies an intermediate position.

Table 6 summarizes the effects of increasing the number of clover units on the vigour and development of a sparse barley crop. Two plants of barley per pot were sown throughout, thus allowing 7·1 l. of root space per plant, while the number of clover units varied from none to eight.

In no case has the presence of the clover increased the yield of a sparse barley crop, nor, on the other hand, has the increasing density of clover caused a serious reduction in the barley yield. In other words, there is little sign that clover can smother barley even when it is present in abundance. But the presence of even a very small amount of barley relative to the clover greatly reduces the growth of the latter (see Table 7).

Table 8 compares and contrasts the effect on a sparse crop of barley of the annual weeds: spurrey, mayweed and chickweed, with clover during its first year, when it acts as an annual. If so grown, all conditions being made as favourable as possible for the growth of the barley, the smothering power of all the weeds is far

greater than that of clover, even though the roots of clover are heavier per unit volume of soil used than those of spurrey and mayweed, and equal to those of chickweed. Table 8 gives the yield per pot with a constant amount of barley but

TABLE 6. *Yield of barley and clover with various densities of clover*

No. of plants per pot		Maximum no. of barley shoots	Yield of barley, g. per pot		Yield of clover (air dry)
Barley	Clover	(tillers)	Grain	Total	g. per pot
2	0	35	41·0	102·0	—
2	1	32	37·1	94·8	18·2
0	1	—	—	—	59·1
2	2	35	39·3	99·6	42·9
0	2	—	—	—	94·1
2	4	35	36·5	92·5	43·3
0	4	—	—	—	121·0
2	6	34	36·3	89·4	62·6
0	6	—	—	—	131·5
2	8	33	38·8	89·2	59·1
0	8	—	—	—	127·2

TABLE 7. *Percentage reduction in barley yield due to presence of clover and of clover due to presence of barley*

No. of plants per pot		Percentage reduction in yield		
		Barley		
Barley	Clover	Grain	Total produce	Clover, total produce
2	1	9·5	7·1	69·2
2	2	4·1	2·3	54·4
2	4	11·0	9·3	64·2
2	6	11·5	12·4	52·6
2	8	5·4	12·5	53·6

TABLE 8. *Comparison of clover and other competitors in a sparse crop of barley*

No. of plants		Spurrey, g. per pot		Mayweed, g. per pot		Chickweed, g. per pot		Clover, g. per pot	
Barley	Weed	Barley	Weed	Barley	Weed	Barley	Weed	Barley	Weed
2 or 4	1	92·0	2·0	95·8	5·8	?	?	94·8	?
2 or 4	2	89·5	4·5(?)	95·4	4·2	46·2	44·5	99·6	42·9
2 or 4	4	90·3	5·6	95·8	7·1	20·0	62·3	92·5	43·3
2 or 4	6	91·0	6·7	82·6	17·8	14·5	64·1	89·4	62·6
2 or 4	8	85·0	10·6	79·5	19·2	8·8	66·7	89·2	59·1

with gradually increasing proportions of weed or clover. With spurrey and mayweed there were 4 plants of barley per pot and the stubbles were not weighed; with chickweed and clover there were 2 plants and the stubbles are included in the weights given.

The clover, it will be seen, has only very slightly reduced the barley produce even when it is in the proportion of 8 plants to 2 of barley, and in this it is comparable

with spurrey and mayweed and totally different from chickweed. When the number of clover plants is increased from 2 to 8, the reduction in the barley crop is only 10·5%, while under similar circumstances chickweed would give 81%. The corresponding figures for spurrey and mayweed were 5 and 17% respectively. In the absence of barley, clover shows an increase from 94 to 127 g. or 35% as the number of plants is increased from 2 to 8 (see p. 113), while in presence of 2 plants of barley it is raised from 43 to 59 g. or by 37%. This would suggest that the effect of the barley is a constant quantity and affects the capacity to grow clover to a similar extent whether the clover crop be thick or thin.

Method of interaction of clover and barley

Kaserer (1911) states that the relationship between the roots of a leguminous plant when mixed with a grass is quite different from that of leguminous plants grown together, or of two gramineous plants grown in association. A careful examination of the mingled roots of clover and barley in the present series of experiments does not confirm these observations. The clover roots were full of nodules and were apparently perfectly normal. They mingled completely with those of barley, yet the roots of the two plants could be pulled apart without much difficulty. There was no sign of attachment of the roots of one plant to those of the other. Both plants provided a large fibrous mass, the clover quite equal in amount to the barley.

When clover was grown alone, the character of the roots changed as the thickness of planting increased. One plant per pot (14·2 l. of root space per plant) sent out thick roots in fair number from the crown, but where the number of plants was greater, there was a distinct tap root from which a number of fairly thick roots came off at right angles.

Table 9 compares the effects of increasing congestion using (*a*) the same species, (*b*) another species.

Table 9. *Comparative reduction in the yield of barley and clover due to increase in the number of barley or clover plants*

	Percentage reduction due to increased barley	Percentage reduction due to increased clover
A. Reduction in produce of 2 barley plants		
(1) With 2 additional barley or 2 clover plants per pot	49·1	2·4
(2) With 4 additional barley or 4 clover plants per pot	64·9	9·3
(3) With 6 additional barley or 6 clover plants per pot	75·5	12·4
B. Reduction in produce of 2, 4 or 6 clover plants		
(1) Two clover with 2 barley or 2 additional clover plants per pot	54·4	35·7
(2) Four clover with 2 barley or 2 additional clover plants per pot	64·2	27·6
(3) Six clover with 2 barley or 2 additional clover plants per pot	52·4	27·5

The effect of increasing the congestion of barley plants on the productivity per plant is very similar to what we have previously found (Mann & Barnes, 1950), but increasing the clover has remarkably little effect on the growth of the barley with which it is mixed, and is of an entirely different order from what we have obtained with the other weeds studied. The presence of barley, as indicated above, reduces in a remarkable manner the growth of clover and the reduction per plant is not widely different whatever the proportion of barley to clover. It is much greater than that produced by increasing the congestion of the clover itself.

Distribution of nitrogen between barley and clover

Whether barley and clover are grown alone or together under the conditions already described, the nitrogen content of the produce is not widely different, as is shown in Table 10.

Table 10. *Nitrogen in barley and clover grown alone or together*

	Nitrogen percentage in dry matter			
	Barley grown alone	Barley grown with clover	Clover grown alone	Clover grown with barley
Grain	1·58	1·64	—	—
Straw and stubble	0·36	0·34	—	—
Roots	1·22	—	3·07	—
Clover above ground	—	—	2·83	3·07

The presence of the clover evidently does not increase the concentration of nitrogen in the barley material, in spite of the fact that the total nitrogen contained in the clover crop is much higher than that in any other crop that we have grown. In our experiments, 750 mg. of nitrogen were added to each pot and the content of nitrogen in the whole crop, including roots, was as shown in Table 11.

Table 11

	mg. per pot
Barley grown alone	882
Clover grown alone	3063
Barley (2 plants) with a mean number of 4·2 plants of clover	2168
Barley (4·2 plants mean) with 6 plants of clover	2605

The clover has evidently gained nitrogen from other sources than the nitrogenous fertilizer added. The effect on the nitrogen content of the barley is shown in Table 12.

Table 12. *Nitrogen in barley in presence and absence of clover*

No. of plants		Total nitrogen in barley crop per pot (mg.)	Reduction with clover (%)
Barley	Clover		
2	0	812	—
2	1	703	13·4
2	2	728	10·3
2	4	662	18·5
2	6	666	18·0
2	8	692	14·8

There is thus a reduction of 10–18 % in the amount of nitrogen in the barley crop above ground when it is grown with clover. The yield of barley is itself reduced in presence of clover by 8·7 % (p. 116). The reduction in nitrogen absorption by two barley plants shows that, at this stage, in the presence of sufficient readily available nitrogen, the effect of the competition of clover is greater than any capacity of that crop to supply additional nitrogen. It appears, in fact, as if the clover actually uses nitrogen which could otherwise be utilized by the barley. This is confirmed by Table 13, giving the results when a variable amount of barley is grown with a thick clover crop.

TABLE 13. *Nitrogen in a variable barley crop in presence of clover*

No. of plants		Total nitrogen in barley crop above ground		
Barley	Clover	Grown alone (mg.)	Grown with clover (mg.)	Reduction with clover (%)
1	6	801	617	23·0
2	6	812	657	19·1
4	6	726	697	4·0
6	6	803	646	19·6
8	6	730	720	1·4

Whatever be the thickness of planting of the barley, the absorption of nitrogen by that crop is remarkably constant and with this thick crop of clover, the mean reduction of the crop due to the presence of clover (14·1 %) is almost identical with the reduction of the nitrogen absorption. In this case, therefore, the barley has been able to get from the other available sources in the soil or from the clover and its exudation, the nitrogen it requires to make up the composition of the crop to its usual value.

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THE CHEMICAL CONTROL OF WATER WEEDS IN THE GEZIRA AREA OF THE SUDAN

I. PRELIMINARY EXPERIMENTS

By K. WILSON JONES, M.Sc. and F. W. ANDREWS, D.Sc.

Research Division, Ministry of Agriculture, Sudan Government

Chlorophenoxyacetate weed-killers have been tested for the control of aquatic weeds in the canals of the Sudan Gezira. Using water in tanks, it was shown that 10 parts per million of sodium 2-methyl-4-chlorophenoxyacetate (Methoxone) or of sodium 2:4-dichlorophenoxyacetate (Fernoxone) would kill most of the angiospermous water-weeds found in the Gezira area.

Small-scale field experiments, using channels of known water capacity, have shown that not only leaves and stems but also seeds and underground rhizomes were killed in treated stagnant water, provided that the weed-killers were applied as dusts. The water remained toxic to crop plants for approximately 7 weeks after treatment.

In a large-scale experiment in 1950, treating normal irrigation canals, temporary weed clearance was attained, re-infestation was delayed, and one major canal, 4·5 km. long, remained weed-free 12 months after treatment.

Introduction

The Gezira, lying between the Blue and White Niles immediately south of Khartoum, has been used for large-scale cotton growing under irrigation since 1925, when the dam at Sennar on the Blue Nile came into operation. The area under irrigation has grown from an original 300,000 acres to nearly a million, one half of which is cropped annually in rotation. Of the cropped area, one half is under cotton and the remainder principally under sorghum with some legumes. The water from the Sennar reservoir, travelling north, passes through a main canal for 57 km., over which distance the water-level is below that of the land. North of kilo 57 the land-level is sufficiently low for irrigation by gravity flow. Thereafter the main canal runs the length of the scheme, branching twice. From it and its branches major canals fan out and in turn feed the minor canals from which the water is run through pipes on to the land. The total length of the canal system is about 4200 km.

The cotton-watering season normally ceases at the end of March. From the end of March until the middle of July the canals are allowed to dry except those supplying domestic water to villages and European houses; these are known as summer-watering and the others as summer-dry. Most of the major canals are summer-watering.

For administrative reasons, watering of the land takes place by day only, and the canals are filled up during the night for watering to recommence the following day. This night-storage produces semi-ponding conditions favouring the growth of aquatic and bank weeds.

Aquatic weeds

The aquatic plants present in the canals have been described by Andrews (1945).

In the early days of the canalization scheme weed growth was negligible; by 1937, however, the rapid increase of the weeds was causing concern and investigations into improved methods of clearance were started. To-day there is so much weed in the canals towards the end of the watering season that available labour is often insufficient to remove it and cotton is sometimes affected by the resulting shortage of water.

Weeds in the Gezira canals are normally hand-removed by gangs of labourers. Where weed growth is light or for the removal of seedlings at the beginning of the season, special rakes with wire netting between the tines are used to dislodge the weeds from the bottom; they are then drawn out by hand. For *Chara globularis*, which will not float when cut, the only method is to drag the weed out with large rakes. An alternative method where weed growth is light is to draw heavy chains wrapped with barbed wire through the canals by means of camels. A third method, especially useful for emergency treatments, is the use of paddle-driven weed-cutting launches carrying large V-shaped oscillating blades. The cut weed drifts downstream and is drawn out of the water by labourers. In the present state of the canals these measures are only palliatives, and before the advent of the so-called hormone weed-killers no practicable method of eradication was known.

Laboratory experiments

In 1946–7 some Methoxone (sodium 2-methyl-4-chlorophenoxyacetate) was supplied by courtesy of Messrs Imperial Chemical Industries Ltd. and used for laboratory experiments.

Preliminary tests showed that sprigs of *Potamogeton perfoliatus* were killed in about 2 weeks by continuous exposure to a solution containing 4 p.p.m. of Methoxone, and in about 3 weeks by exposure for 2 days to a solution containing 10 p.p.m. of Methoxone, followed by transfer to water.

Further experiments were carried out using plants rooted in soil at the bottom of large dishes. These experiments showed that the soil interfered in some way with the effect of the Methoxone on the plants, for concentrations less than 8 p.p.m. were ineffective. However, it was established that the three commonest canal weeds, viz. *Najas pectinata*, *Potamogeton nodosus* and *P. perfoliatus* were killed after not less than 3 weeks' exposure to a concentration of 10 p.p.m. in the presence of soil. Of these the most resistant was undoubtedly *P. nodosus*. The chemical was added as a water solution, as a powder, and as a diesel oil solution, without significant difference in the result.

Fernoxone (sodium 2:4-dichlorophenoxyacetate), also kindly supplied by Messrs Imperial Chemical Industries Ltd., was also tested and gave substantially the same results as Methoxone.

Small-scale field experiments

To test these chemicals on a larger scale, the channels of an experimental system, each 50 m. long with a cross-section of about 1 sq.m., were used. All received water from a supply canal and were very full of weed at the time the experiment commenced.

Exp. 1. The volume of the water in one channel to be treated having been ascertained, banks were constructed across the middle of this and of another (control) channel to divide each into two sections. The calculated dose to give a concentration of 10 p.p.m. was then added on 29 May 1947 to each section of the treated channel in the form of a 1% Methoxone dust with a lime diluent. The portion of each of the two channels nearest the supply canal was periodically refilled during the following weeks as the level fell by evaporation, but the portion cut off by the bank was allowed to dry out. These two portions of the treated and control channels simulated summer-watering and summer-dry conditions respectively. Weeds at the time of treatment consisted of *Potamogeton nodosus* and *P. perfoliatus* with *Najas pectinata* and *Ottelia alismoides*.

Fourteen days after treatment the channels were re-examined. Apart from a certain browning of the weeds in the treated channel and a considerable drop in the water-level of both the summer-dry sections, there were no very striking differences to be noted. By 23 June (25 days after treatment) all the weeds in both sections of the treated channel had disappeared, leaving only a black sludge on the bed of the channel. In addition, the fringe of nut-grass (*Cyperus rotundus* L.) on the banks of the treated channel was brown and the leaves and stems were dry and tended to break up at the original water-level. Samples of treated and control water taken at this date were used to water pots sown with sorghum. Germination and growth were alike adversely affected by the treated water. A similar result was given by treated water sampled on 6 July, by which date both sections of the treated channel were completely free from living aquatic weeds or living nutgrass.

The treated water was sampled again on 9 August, when it produced no effect on the germination of the sorghum; nor was any effect produced by the remains of the sample taken on 23 June, which had been stored in bottles until 9 August. It is possible that some form of bacterial action is responsible for the breakdown of the Methoxone in solution.

The summer-dry portions of the channels were refilled on 9 August by breaching the central banks, the water being allowed to run through them on to small plots sown with sorghum and cotton to ascertain if any toxic effect remained. No differences in germination or subsequent growth of either of these crops were noted between plots watered from the treated and control channels. After 3 weeks, seedling weeds were appearing in the previously dried-out portion of the control channel, but both portions of the treated channel remained clean until August 1948, suggesting that not only the weeds but also their seeds had been killed by the treatment.

Exp. 2. In 1948 the effect of the application of Methoxone and Fernoxone in water solution was tested on the aquatic weeds and on the subsequent growth of cotton and sorghum crops. A replicated experiment was carried out using eleven channels similar to the two used the previous season (Exp. 1). Each channel was divided into two portions as before but, whereas in 1947 the summer-watering portions were filled up by pipes as required, in 1948 they were left open to the supply canal for the first 3 weeks after application of the herbicide. As in 1947, the weeds present were *Potamogeton perfoliatus*, *P. nodosus*, *Najas pectinata* and *Ottelia alismoides*.

Treatment was carried out on 23 April, roughly corresponding with the normal date of cessation of irrigation in the Gezira. The herbicides were sprayed on the water as the sodium salts, 10% for Methoxone and 2% for Fernoxone, so as to give a final concentration of 10 p.p.m. in the channel. The summer-watering portions were thereafter kept filled and the summer-closed portions allowed to dry. On 27 July the contents of five of the summer-watering portions were first used to water five sorghum plots and then, after refilling of the summer-closed portions of the same channels, five more sorghum plots were watered from them. A similar set of twelve cotton plots was watered from the two portions of each of the six remaining channels on 15 August. 27 July and 15 August are about the average sowing dates for sorghum and cotton respectively in the Gezira. As no significant differences were shown between the yields of plots watered with Methoxone, Fernoxone or untreated water, or with water which had passed through channels previously treated with either weed-killer, it seemed that treatment carried out in April was harmless to these two crops when sown about their usual sowing date.

The killing of the aquatic weeds with the liquid treatment was disappointing, being never complete except in the summer-dry portions of the channels. Even here it was doubtful whether the herbicide or the drying caused the effect. In the summer-watering portions the weed was at first heavily reduced, but a considerable amount survived, especially *Potamogeton nodosus*. On 30 November 1948, the average infestation, on an arbitrary scale of estimation, was:

	Summer-dry (%)	Summer-watering (%)
Control channels	70	80
Methoxone channels	20	75
Fernoxone channels	33	70

The use of solutions of the chemicals proved therefore less effective than the use of the powder in the previous season, though the open communication of the treated channels with the supply canal may have accounted for some loss of strength by dilution. In the summer-dry portions, however, where no mixing could have taken place, the kill was also not complete.

Exp. 3. In 1949 Methoxone, as a 5% powder with a lime diluent, was tested, as well as a home-made dust containing 5% Fernoxone, 10% slaked lime and fine river silt as diluent. It was suspected from Exps. 1 and 2 above that the dust containing lime might be less soluble than the sodium salts alone, and might owe its superiority to the deposition of the weed-killer on the plants from which a concentrated solution entered the leaf tissues. The presentation of both compounds in the presence of calcium salts permitted a straightforward test of the intrinsic toxicity of the two chemicals.

Plants of the same aquatic weeds as in the previous experiments were sown in twelve experimental channels and the volumes of the channels subsequently measured. By the end of March 1949 weed growth approximating to complete water-block existed in all channels. Four channels were selected for each of the two treatments and four were left as controls. The water-level of the channels was maintained by opening pipes from the supply canal when required.

The weed-killers were applied on 3 May 1949 to each channel to give a concentration of 10 p.p.m. of the pure substance. By 4 June all aquatic weeds had disappeared from the treated channels. The presence of weed-killer in the water was tested weekly by using small samples to water pots sown with sorghum. For the first 3 weeks the growth of the sorghum was completely inhibited, but the effect then decreased, becoming imperceptible at the end of 7 weeks. The pipes from the supply canal were then left open until 6 July 1950. Up to the end of the experiment only two patches of weed appeared in the treated channels—one in each of the treatments—and in both there was strong evidence that the reinfestation had occurred through fragments of weed being carried in from the infested supply canal. One of the control channels of this experiment was treated on 6 October 1949 with a mixture of Fernoxone in river silt containing 5% active agent but no lime. The principal aquatic weed was *P. nodosus* which was all killed, but more slowly than in the previous treatment. No living weed was found after 19 November. The slowness of kill may be a seasonal effect.

It appeared from these small-scale field experiments that the hormone weed-killers showed great promise for the control of water weeds, and that the powder formulations were, at least in the field experiments, more effective than the liquid spray applications.

Large-scale field experiments

In 1950 a large-scale application of Fernoxone in the Gezira canals was carried out. Fernoxone was chosen principally because it was the cheaper chemical. Two areas were selected, one at Hag Abdulla in the south and the other at Turabi in the north of the scheme.

In the Hag Abdulla area, a compact system of canals fed by pumps from the main canal included both major and minor canals, and totalled approximately 50 km. It was chosen on account of (*a*) the clean condition of the main canal above the

point of off-take, ensuring a minimum of reinfestation from the supply, and (*b*) the small amount of silt-clearance and therefore weed disturbance scheduled to take place during the 1950–51 season.

At Turabi, in the north, where the weed growth is normally the heaviest in the system, it was impossible to locate a compact area with the advantages found at Hag Abdulla; the main canal is itself infested at the point of off-take, and many of the canals were due for silt removal. Accordingly, portions of several canals which were badly infested but not due for silt clearance were chosen as spot tests. The total length treated in this area was 24·5 km., including one length of a small major canal.

The standard application consisted of 10 p.p.m. of Fernoxone, applied after dilution with river silt to contain 5% of the active agent. In one section of the Hag Abdulla area, however, a modified mixture containing 10 % of slaked lime was employed over about 14 km. of minor canals. The mixture was prepared at two convenient sites by churning the materials in empty oil drums. Precautions were taken to prevent accidental damage to cultivation through wind drift. The canals were measured in March 1950 to determine the volume of water which would be present in the canal when watering ceased. The correct amount of mixture was then placed ready for application in 50 kg. bags at equal distances along the length of the canals. In no case was the error in volume measurement believed to be greater than 10 %—i.e. the concentration actually applied was between 9 and 11 p.p.m.

The normal watering of the cotton crop ceased on 31 March and application of the mixture commenced in the two areas simultaneously on 1 April as soon as water flow had ceased and the pre-determined static water-levels had been obtained. The application was carried out by labourers walking in the water or in the larger canals by men pulled slowly on rafts. The application was completed in Turabi by 3 April and in Hag Abdulla by 5 April. In the latter area it was noted on the morning of the 6th that plants of *P. perfoliatus* in the first-treated canals were losing turgidity and that many of the flower stalks lay limp on the surface. Observations were thereafter carried out at convenient intervals.

(*a*) *Hag Abdulla observations.* The original weeds in this area, in approximate order of importance at the time of application, were: *Najas pectinata*, *Potamogeton nodosus*, *Ottelia alismoides*, *Vallisneria aethiopica*, *Chara globularis* and *Potamogeton perfoliatus*.

By 14 April, 2 weeks after the application was commenced, all weeds with the exception of *Chara globularis* and *Potamogeton nodosus* were seen to be breaking up and dying. The water was brown, with floating masses of weed fragments, finely divided pieces of *Najas pectinata*, detached and bleached leaves of *Ottelia alismoides*, and brown detached leaves of *Vallisneria aethiopica*. As the debris decomposed and sank, large hitherto unsuspected beds of *Chara globularis* were revealed at the bottom of the canals, and by 21 April these seemed to be increasing. They continued

to grow until the canals dried out, or until the arrival of the silt-laden flood water checked further increase. The increase was almost certainly aided by the withdrawal of competition from the angiospermous weeds.

As the decomposing plant debris made the water highly objectionable as a domestic water supply for the area, it was decided to drain the treated canals and to refill them with fresh water. This was done and refilling commenced on 24 April. It was hoped at that date that the killing of the weeds and seeds would have progressed sufficiently far to ensure a lasting effect. Up to 14 July no weeds remained in any canals of the summer-watering set, except for widespread patches of *Chara globularis*. Water was opened into the summer-dry canals during the ensuing week.

Weed seedlings began to appear in all canals about 5 August, irrespective of whether they were summer-dry or summer-watering canals. Infestation began at the water's edge and many seeds had obviously survived the treatment. These seedlings were raked out, but the regrowth continued vigorously. By September it became clear that the cleanest canals were the summer-watering ones, but it was also obvious that the draining of the canals in the previous April had seriously interfered with the kill of seeds. No differences were observed between the canals treated with the simple and with the lime mixture, but the 11 km. of major canals in this area showed a much smaller regrowth of weed than the rest. There did not appear to be preferential survival of any particular weed; the proportions were much the same as had been recorded before treatment though the actual numbers were smaller.

(*b*) *Turabi observations*. The weed flora in this area differed somewhat from the above, with weeds in order of importance as follows: *Potamogeton crispus*, *P. perfoliatus*, *Chara globularis*, *Potamogeton nodosus*, *P. pectinatus* and *Najas pectinata*.

The weeds in these canals behaved under the influence of the treatment more or less as those at Hag Abdulla. No drainage of the treated water was however carried out in this area, as, the treated canals being scattered, alternative sources of water were available to the inhabitants. The canals were filled with fresh water 3 weeks after the application of the herbicide, to supply cotton pickers working on the last of the crop, and then allowed to dry. By 25 May all were dry except for two lengths of which one was a major and the other a minor canal. When water was supplied in July the fringe of nutgrass immediately showed signs of recovery from the roots, indicating that these at any rate were unaffected. With the exception of floating debris from the upstream canals, the treated canals all appeared clean. By 16 August, however, seedlings appeared in two of the canals, mainly towards the water's edge, and a weeding chain of the type described above was used to dislodge them. This was repeated 2 weeks later, after which no further seedlings made their appearance. A thorough inspection in early October, made with rakes so as to discover any seedlings, failed to bring any to light, except for a heavy infestation of *Najas pectinata* in the tail of one canal; this latter infestation was probably due to rooting of washed-down fragments. By the end of October it was found that the

tail of a neighbouring treated canal was similarly infested. These infestations appeared to spread upstream during the following month, and by 15 November it was noted that of a total of 24·5 km. of canals treated, 21 were completely free of weeds. The principal weed in the infested tails was *Najas pectinata*, with occasional *Potamogeton nodosus*.

Regrowth of weed continued in late November, when *P. crispus* first began to appear among the other weeds in the infested tails, again spreading upstream. At the end of the month a total of 5 km. was infested, though most of it lightly. Two chainings were given, and no further spread was observed in December.

Regrowth in treated canal sections other than the two whose tails had been infested earlier, occurred in January 1951, when a further 8·2 km. became infested in two canals, one with *P. perfoliatus* and the other with *P. crispus*. By the end of the month 61% of the total length treated had become re-infested. During February and March the infestation spread in all the treated canals with the exception of the small major canal referred to above. The entire length of this major, 4·5 km. approximately, remained clean throughout, up to the end of March 1951. This represented 18% of the total length treated at Turabi.

An examination of the canals supplying the treated lengths showed that these were all clean, and a comparison of the distribution of the various weed species within the treated lengths showed that the re-infestation was almost always by the species originally present. As, in particular, one treated length was dominated by *P. nodosus* and an adjacent treated length was dominated by *P. crispus*, in March 1950 and each became re-infested in January 1951 with the same weed, it is difficult to ascribe the regrowth of these weeds to washing-down of seeds or weed fragments. In view of these facts it is considered highly improbable that the heavy regrowth of January 1951 could have occurred as an introduced re-infestation, and it must be ascribed to an incomplete kill of either seeds or rhizomes. It is, however, noteworthy that reappearance of the weeds was greatly delayed in all canal lengths as a result of the treatment.

Discussion

The apparent eradication of the aquatic weeds from the treated canals up to the middle of December 1950 gave promise at first that a solution of the problem was at least in sight. These hopes have not been fulfilled, and regrowth has occurred over much of the total length of canals treated. For the reasons given above, it seems as if the re-infestation is due to seed which has lain in the canal bed and for some reason escaped the action of the herbicide.

It remains to consider the reasons for the differences in results between the highly successful small-scale experiments and the less successful large-scale ones. Leaving out of consideration the regrowth at Hag Abdulla, for which the reasons seem fairly obvious, the differences are rather obscure. A possible explanation is that, although the herbicide was added to the water at the end of the cotton-

watering season, a further filling had to be given to some of the canal lengths in order to supply cotton pickers working in areas where no other water was obtainable. This doubtless resulted in a certain amount of washing downstream, and it is noteworthy that most of the January 1951 regrowth took place over such lengths. On the other hand, some regrowth occurred in one length which was not so refilled, and no regrowth occurred in the treated major canal through which clean water was passed for the refilling. In the small experimental channels, periodical refillings were given to maintain the water-level; these apparently did not interfere with the effectiveness of the treatment, but the amount of washing-down taking place in a 50 m. channel and a 5 km. canal are hardly comparable.

In preparation for experiments in the 1951–2 season, measurements of the canals were carried out more accurately than in the previous season. Cross-sections of canals, taken at intervals of 100 m., instead of 500 or 1000 m. as in the previous season, showed great and hitherto unsuspected variations in the shape and volume of the canal, and in a few cases a difference of 50% or more in the cross-section between two points 100 m. apart. Whilst errors over the whole length treated may be expected to balance out, it seems reasonable to surmise that patches of weed, seed and rhizomes may have survived in the deeper pockets of a canal bed, where the strength of herbicide may have fallen to as low as 7 or 6 or even 5 p.p.m. of water.

It seems, on balance, that a good deal of the regrowth can be ascribed to washing of the herbicide downstream at the time of canal refilling (where such refilling occurred), coupled with random variations in the strength applied due to irregularities in the canal bed.

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VARIATION WITHIN FRENCH BEAN VARIETIES

By A. J. BATEMAN, Ph.D.

John Innes Horticultural Institution, Bayfordbury, Hertford, Herts

(With 2 Text-figures)

The internal variability of several varieties and strains of French bean is investigated, confining attention to the single character, seed weight. Out of eleven strains examined only two appeared to be homogeneous for seed weight, whilst two were obviously heterogeneous even by superficial examination of the seed.

A comparison of the distribution of seed weights in five strains of Canadian Wonder showed that each strain contains at least two or three lines out of the minimal estimate of four shared between them.

The natural out-crossing of fourteen varieties, including those analysed for seed weight, is compared. There are indications that varieties vary in their out-crossing propensities. No indication was found of the causes of the high frequencies of out-crossing occasionally encountered.

The possible sources of intra-varietal variation are briefly surveyed.

Introduction

The study of intra-varietal variation in the French bean (*Phaseolus vulgaris*) is no novelty. The pure lines used as the basis of Johannsen's classical experiments (1903 and 1909) were all obtained from a commercial variety, the brown Princess Bean. The lines were selected out on the basis of seed weight for which the extremes had a ratio of about two to one.

However, the impact of Mendelism on plant breeding, especially through the importance attached to varietal purity, might have been expected, in the intervening half-century, to have increased the uniformity of French bean varieties, for this species is overwhelmingly self-fertilizing, and it should therefore be a simple matter to establish pure lines.

To test the uniformity of present-day varieties Johannsen's method of extracting pure lines has been repeated in a representative selection of horticultural varieties and, in particular, in Canadian Wonder. This variety was specially chosen because several seed merchants market more than one strain of it, an indication that there was likely to be much internal variability.

At a casual glance, a sample of seed of most varieties appears to be very uniform. There is certainly great uniformity in the colour and markings of beans of one variety, in spite of the wide variation within the species as a whole. This is undoubtedly because *obvious* heterogeneity in a variety, especially of the seed itself, has a bad psychological effect on customers.

Seed weight, like seed colour, is of negligible horticultural value, but since any sample will show great non-heritable variation in this character, additional heritable

variation is likely to pass unnoticed. Consequently, it is unlikely to have been subject to deliberate selection, and heritable variation in seed weight should provide a sensitive test of genetic variability within varieties.

It should be emphasized here that heritable variation in seed weight is almost, if not entirely, maternally determined; that in fact seed weight is a measure of the genotype of the seed parent rather than that of the individual seeds. Heritable variation can therefore only be detected by a comparison of the mean seed weights of mature plants and not by a comparison of different seeds borne in a single plant.

Results

Samples from thirteen lots of seed of seven varieties were sown in 1946. The strains were obtained from four seed merchants, who may be referred to as A, B, C and D. In this paper the letter designating the seed merchant will precede the name of each strain. Each sample was sown in two rows of twenty seeds, one in each of two randomized blocks. In only one of the thirteen samples, C Unrivalled, was there a marked variation in seed colour. In this, whilst most of the seeds were long, white and with a dark brown ring round the hilum, 8 % were similar in size but entirely white and flattened, and 2 % were small, round and white. These aberrant colour types were assumed to be admixtures of other varieties and were extracted from the sample before sowing. The other samples were random. There was one other obvious case of heterogeneity, in D Canadian Wonder. About 20 % of the seeds, though normal in colour for the variety, were small, round and slightly shrivelled as though harvested prematurely. These were taken to be *bona fide* members of the variety even if aberrant, and were sown with the rest.

The seed quality of the various lots is shown by the number of plants harvested. One variety, A Superlative, completely failed to germinate. There were only four plants of B Prince to harvest, so this also was not tested further. The others were represented by varying numbers of plants as will be seen from Table 1.

In Table 1 the 10-bean weights of only those plants selected as parents of the second generation are given. In estimating means and mean squares for the first generation only those plants producing at least ten properly ripened seeds without split testas were included.

The author's experience with seed weight in the French bean has shown that in pure lines there is a very close relationship between standard deviation and mean. The ratio of standard deviation to mean (often called the 'coefficient of variability') has therefore been taken as the best basis for comparison of the variability of several strains. If the coefficient for non-heritable variability is constant throughout, the magnitude of the overall coefficient indicates which strains contain most heritable variation and which least (these may be pure lines). Using this criterion we can conclude from Table 1 that of the twelve strains surveyed, the two strains of The Prince and C Unrivalled (after extraction of aberrant colours) were most nearly pure lines, all the remainder, especially D Canadian Wonder, carrying appreciably more

variability. Such an approach can only give a rough indication of internal heritable variation. It is necessary, therefore, to pursue the study further.

Up to ten plants from each strain were selected as being representative of the range of variation within that strain, and were used as seed parents of the families of the second generation. Random choice of the parent plants would have been less sensitive in detecting (the aim was detection rather than estimation) the variability present in not more than forty plants.

The total number of parent plants selected was eighty-seven. Each had its seed divided into three lots which were sown in each of three contiguous randomized blocks. Each plot consisted of five plants. The plants grew 1 ft. apart each way and a guard row surrounded the whole.

The seed had been obtained by natural pollination, so some hybrid seed might have been present. Though this might increase the variance between families it would also increase the estimate of error. It would not therefore lead to over-estimation of the heterogeneity in the original variety.

An analysis of variance of the 10-bean weights was carried out separately on each strain, and the mean square for error compared with the difference between families. The variance ratios for these statistics, together with their level of probability are given in Table 1. The expression 'V. small' is used where the probability is much less than 0·001, and 'high' when it is much higher than 0·2. The conclusions are in good agreement with the results of the first generation shown in the same table.

A The Prince, D Continuity and C Unrivalled appear to be pure lines, at least in respect of seed-weight characters. All the other strains show more or less internal variation. The nature of this variation is best brought out as a diagram (see Fig. 1). With C The Prince, there seems to be a contradiction between the coefficient of variability, which indicates a pure line, and the variance ratio which indicates heterogeneity. The coefficient is, however, based only on a comparison of single plants and is not separable from non-heritable variation, whereas the variance ratio is based on a comparison of families of fifteen plants in three replications and takes account of non-heritable variation. Much more weight attaches therefore to the latter statistic.

The series of strains of Canadian Wonder are the most informative. It is tempting to speculate on whether these heterogeneous strains are a mixture of a small number of distinct pure lines or whether a large number of genotypes are represented with perhaps a continuous distribution. With a maximum of ten lines from each strain the data cannot be more than suggestive.

However, the distribution of the ten lines of A ordinary stock, when compared with the standard error of line means, suggests that there must be at least three distinct lines with means of 5·5, 6·3 and 7·0 g. approximately. C Improved strain shows the same distribution, though with a lower error variance so that more significance is attached to the spread, and might contain the same three lines. A Selected strain appears to have the first two lines only. It could therefore have

TABLE 1. *Comparison of the variability of* 10*-bean weight* (*g.*) *of various strains and varieties*

Variety ...	Canadian Wonder										The Prince				Perpetual		Black Wonder		Continuity		Unrivalled	
Strain ...	A		A Selected		C Improved		D		B Reselected		A		C		B		C		D		C	
First generation																						
No. of plants	15	15	11	12	17	13	11	7	9	6	13	17	15	15	10	11	18	13	17	12	7	5
Mean	6·54	7·87	6·23	5·85	6·54	6·64	4·49	6·14	7·24	6·95	6·44	7·16	6·78	6·87	3·73	3·38	4·37	5·15	3·69	3·90	6·01	5·80
Mean square (pooled)	1·05		0·74		1·07		4·94		3·59		0·24		0·33		0·36		1·51		0·43		0·18	
S.D./mean	**0·146**		**0·141**		**0·149**		**0·444**		**0·254**		**0·086**		**0·072**		**0·169**		**0·255**		**0·181**		**0·074**	
Values of parents of 2nd generation	8·30	8·95	5·50	5·90	7·10	8·25	7·25	5·75	-		7·00	7·00	8·30	7·60	4·50	3·45	4·75	6·90	4·70	4·00	5·55	5·25
	5·25	9·40	7·10	6·25	4·95	6·05	1·55	6·45	—		7·00	6·80	6·20	6·70	3·05	4·05	6·20	5·50	2·90	4·80	5·75	7·45
	7·65	5·75	6·30	5·30	9·95	7·50	1·70	6·35	—		5·75	8·30	7·10	6·60	4·00	2·65	5·40	5·35	4·25	3·25	6·60	5·10
	6·00	7·30	7·40	4·90	7·85	6·80	6·20	—	—		6·50	6·25	6·25	7·00	3·40	3·00	2·75	2·95	3·85	4·25	—	—
	7·75	6·80	—	5·15	6·80	—	—	—	—		6·80	6·40	5·75	—	—	—	2·80	—	2·50	3·55	—	—
Second generation																						
Variance ratio, families/error	7·52		6·13		11·98		39·02		—		1·43		9·54		8·35		13·70		1·85		2·18	
Degrees of freedom	9/18		8/16		8/16		6/12		—		9/18		8/16		7/14		8/16		9/18		5/10	
Probability	**<0·001**		**>0·001**		**V. small**		**V. small**				**High**		**V. small**		**V. small**		**V. small**		**<0·2**		**<0·2**	

been derived from their ordinary stock by elimination of the largest seeded lines. Since it still contains the remainder of the variability it is doubtful whether it arose as a single plant selection.

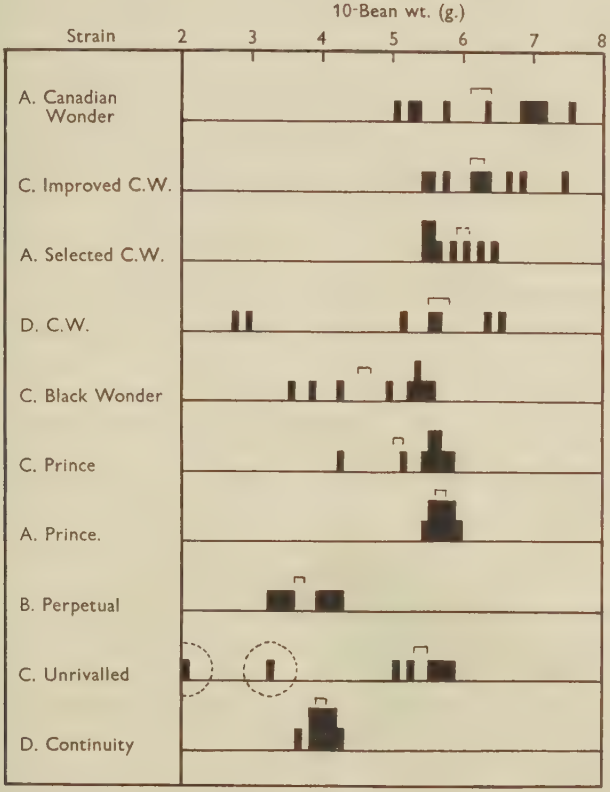

Fig. 1. The distribution of line means within strains. Each black column of unit height and 0·1 g. wide represents one line. Columns of double height represent two lines of closely similar means. The horizontal mark above each series of lines represents by its span the standard error of the estimates of line means. A pure strain would ideally be represented by a normal curve with this standard error as its standard deviation. The two inset columns in Unrivalled represent the approximate means of the aberrant colour types excluded from the trial.

D strain appears to contain the same two lines with, in addition, an extremely aberrant type with a mean weight of about 3 g. These plants had the runner tendency, produced pods in proportion to the seed size and were late maturing. They were therefore generally undesirable. Since they could have been easily identified and as easily eliminated it is strange that they should have persisted and formed as much as 20 % of the seed numerically and 10 % by weight. Of the

other strains, C Black Wonder comprises at least two lines. So also does C The Prince though A The Prince is very uniform with a seed weight equal to the bulk of C strain. The small size of the samples does not permit us to attach any significance to this difference. B Perpetual clearly has two seed weights. The difference between the two classes is not in this case confined to seed weight. Dr Sismanidis, working at this Institute, had earlier separated out two lines from this same variety, though his selection was based not on seed weight but on earliness. The early selection had a seed weight corresponding to the lower weight class shown in the figure. This strain might therefore have consisted of two lines, early with smaller seeds and late with larger seeds. There is a suggestion of bimodality about C Unrivalled though the variance ratio is not significant at the 5 % level. It will be remembered that this variety contained other colour types whose seed weights are also represented for comparison.

To summarize the results of the survey: of the ten strains grown for the second generation only two, D Continuity and A Prince, show apparent uniformity for seed weight, whereas two, D Canadian Wonder and C Unrivalled, show extreme variation, comparable to a mixture of varieties. All the variable strains show evidence of being a mixture of at least two, sometimes three, distinct lines, though the possibility of continuous variation for seed weight cannot be ruled out for all strains. In several cases variation in seed weight is associated with other characters such as seed colour, earliness, and plant habit.

Origin of variation

There are many ways in which such variation might arise. New varieties generally originate from varietal hybrids. From the F_1 onwards the heterozygosis will be halved in each generation. By F_6 the heterozygosis of individual plants will be, on the average, $\frac{1}{32}$ of the F_1. If a new variety were based on all the progeny of a single F_6 plant it could still contain appreciable variability. A rule-of-thumb plant breeder might even base a variety on several F_6 plants which were similar in such characters as seed colour, habit, pod shape and earliness (in that order) and not too dissimilar in seed weight. The internal variability of a variety with such a multiple origin would be much greater. To maintain such variability indefinitely the mother seed of the variety would have to be based on a very large number of plants, and recourse even once to single plant selection would eliminate at one stroke all that variability, unless of course the plant were heterozygous.

Mutation could conceivably account for the variation though its importance would depend on the rate. Of this we have little evidence.

Another source of variation is cross-fertilization. Here it is appropriate to consider some data on cross-fertilization in the French beans collected for a number of current varieties grown under British conditions.

Cross-fertilization

The first reference I have been able to find to this subject is Darwin (1857). That author was confused by the use of the term 'kidney bean' to cover French bean and runner bean, two species with quite different degrees of cross-fertilization. Whereas the French bean sets seed freely when bees are excluded, the runner bean fails to set under those conditions. The flowers of the two species appear identical in structure except for the location of the stigma on the style. As a consequence, whereas in French bean, self pollen is deposited on the stigma of an untouched flower, in the runner the keel has to be depressed before self-pollination can occur. The species is therefore normally dependent on insect visitors for seed setting, with the inevitable concomitant cross-pollination.

In French beans, on the other hand, the frequency of cross-fertilization is generally very low, around 1 % (Kristofferson, 1921; Schiemann, 1921), though Barrons (1938) found that in Alabama the frequency was nearer 10 %. The cause of this difference is not known and may be due to the pollinators or to some effect of climate. High frequencies of cross-fertilization do sometimes occur sporadically elsewhere. Darwin (1858) cites two instances in Britain. There seems to be no published work, however, on the normal incidence of cross-fertilization under British conditions. The following account supplies this information.

The experiment utilized the fact that the gene for black seed which is present in the varieties Black Wonder and a black-seeded foot-rot resistant strain kindly supplied to this Institution by Mr L. Ogilvie of Long Ashton is not only dominant but is recognizable in the F_1 seedling by purple mottling of the cotyledons which can be detected before the cotyledons emerge from the soil, and a purple colouring to the hypocotyl just above ground-level. The proportion of crossing of any variety to a black-seeded variety can thus be scored within a week or two of sowing the seed of the plants exposed to contamination. Plots were laid out in such a way that single plants of several varieties were surrounded by plants of one of the black-seeded varieties (see Fig. 2). The plants were grown 1 ft. apart each way. Previous experiments (Bateman, 1947) have indicated that the majority of crossed seed on a plant is fertilized by pollen from immediately neighbouring plants (in this arrangement, all black-seeded), and even in the plot as a whole the ratio of black-seeded to other plants was more than three to one. In this way the frequency of seedlings with purple hypocotyls would represent very nearly the total out-crossing of the plants, while many varieties could be tested simultaneously in a small space.

The amount of crossing is liable to vary with the degree of coincidence of the flowering time of the contaminating and contaminated varieties. Black Wonder is an early flowering variety, whilst Mr Ogilvie's strain is late. The propensity of a variety to outcross can only be decided by testing it against both black-seeded forms. The data were collected over several seasons up to 1950.

There is obvious variation in propensity to crossing, but since it rarely rises to

more than 1 % it is difficult to make statistically significant comparisons between any given pair of varieties. Comtesse de Chambord is outstanding in showing extremely low crossing. No crossing in 4934 seeds corresponds to a maximum frequency of less than 0·1 % at the 2·5 % level of probability (Stevens, 1942). The two other varieties showing no crossing among over 1000 seeds are Granda and Leviathan, both white seeded varieties like Comtesse.

During the accumulation of the above data an attempt was made to discover what environmental factors, if any, caused sudden increases in out-crossing, such as were reported by Darwin and for which the present author has also found evidence, though never in the material contributing to Table 2.

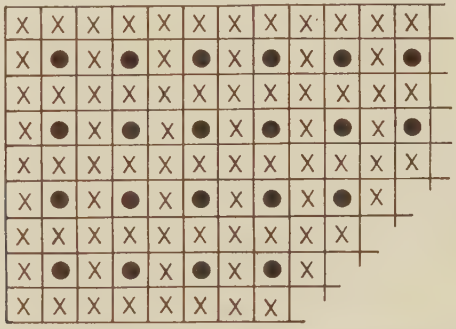

Fig. 2. A diagram of part of the plot for studying cross-pollination in French bean. Each square represents a single plant, spacing being 1 ft. each way. The crosses are black-seeded plants, the circles, plants of the tested varieties arranged in random order.

TABLE 2. *The percentage natural crossing of French bean varieties with two black-seeded forms*

	Contaminating variety						
	Long Ashton strain (late)			Black Wonder (early)			
Tested variety	No. tested	No. hybrids	%	No. tested	No. hybrids	%	Remarks
Canadian Wonder	1256	7	0·6	185	1	0·5	Crossing with both early and late varieties
Lightning	456	3	0·7	291	1	0·3	
Superlative	188	2	1·1	589	8	1·4	
Sunrise	256	2	0·8	435	0	0·0	Crossing with late variety only
Perpetual	1012	4	0·4	403	0	0·0	
Prince	300	0	0·0	402	9	2·2	Crossing with early variety only
Masterpiece	229	0	0·0	246	4	1·6	
Brown Dutch	203	0	0·0	252	1	0·4	
Unrivalled	138	0	0·0	267	11	4·1	
Premier	—	—	—	184	3	1·6	
Comtesse de Chambord	3227	0	0·0	1707	0	0·0	Crossing with neither
Granda	586	0	0·0	429	0	0·0	
Leviathan	426	0	0·0	722	0	0·0	
Continuity	330	0	0·0	528	0	0·0	

The only clue obtained so far has been the discovery of what was in effect male sterility in plants grown in a very hot humid glasshouse. The male sterility here arose through premature germination of pollen in the anther, the tubes forming a tangled mass. It is interesting to note that Darwin (1858) suggested that sporadically induced male sterility might be the explanation of occasional high out-crossing in the French bean.

Following up this clue half the plants being grown in two seasons for the cross-pollination experiment were watered twice daily during flowering, to increase the humidity of the atmosphere. The results were negative.

Discussion

Genetical variation within a strain can exist in two rather different ways. A variety may be uniform except for a small minority of aberrant individuals arising by mutation or contamination. This is economically unimportant. Alternatively, the variety may show continuous variation or discontinuous variation into a few classes of comparable frequencies. The variation then becomes part of the structure of the variety. Since the above described tests for internal variation were based on at most forty plants, usually much less, and two or more groups of comparable frequencies were detected in most strains, it is the second type of variation which pertains to French bean strains. Accidental crossing or mutation could in themselves account for such a situation only if either or both were at a very high level, and since crossing usually produces F_1 plants with a new seed colour these would be speedily rogued out.

The most plausible explanation seems to be that new varieties, which probably arise as descendants of hybrids, are 'fixed' by the breeder before they have achieved homozygosis for genes affecting seed weight. Since so much importance is attached to seed colour it is even possible that separate lines of descent with similar seed colour may be pooled in constructing the new variety. Even so, resort to single plant selection must be very rare, for however wide the base of the variety may have been in the past, one single plant selection in self-pollinating French bean would immediately convert it into a pure line. If a variety consisted of a mixture of lines, out-crossing to the extent of 1 % would allow a certain mingling of the lines with a resultant tendency to continuous variation. Under normal conditions, however, natural out-crossing between varieties is likely to be of less importance as a source of intra-varietal variation than as a source of new varieties.

The presentation of this paper is not to be taken as advocacy of maximum purity of strains of horticultural crops. It has no contribution to make to the issue of uniformity versus diversity in varieties.

The author wishes to acknowledge the work of Miss S. Mann in carrying out the experiment on out-crossing to 'Black Wonder', and the financial assistance of the Agricultural Research Council.

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(*Received* 28 *June* 1951)

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

General Meeting of the Association held on Friday, 12 October 1951, in the Meeting Room, Royal Entomological Society of London, 41 Queen's Gate, London; Dr V. B. Wigglesworth in the Chair.

After the formal business, the following papers were read and discussed:

Insect physiology in relation to applied biology

1. Introduction by the Chairman.
2. Insects in relation to the accumulation and adherence of insecticides. By Dr W. A. L. David.
3. The role of cuticle and egg-shell membranes in the penetration of insecticides. By J. W. L. Beament.
4. Studies on the physical and biochemical action of insecticides. By Dr C. Potter, Dr K. A. Lord and Dr A. H. McIntosh.
5. Features of the ecology and control of the sheep tick, *Ixodes ricinus* L., in Britain. By Dr A. Milne.
6. The developmental cycle of the sheep tick. By Dr J. Allan Campbell.
7. Aspects of the physiology of the sheep tick. By Dr A. D. Lees.

INTRODUCTION

By V. B. Wigglesworth, *Department of Zoology, University of Cambridge*

The view is commonly put forward that in order to advance applied entomology we need to know much more about insect physiology. But if you ask precisely what contribution physiology does make to insect control, you do not in my experience get any very satisfactory answer. The reason for this, I think, is simple enough: insect physiology makes no direct contribution to applied entomology. None the less its influence is, I believe, of considerable importance.

Insects are controlled either by biological methods, by altering their environment in one way or another so as to render it less favourable for the insect pest, or by chemical methods, which kill the insect directly. Biological methods are based upon ecology. They are, in fact, applied ecology. But the proper understanding of the ecology of a pest is impossible without a knowledge of the special physiology of the insect in question, and that cannot be usefully studied without a pretty good knowledge of insect physiology in general.

The great value of the study of physiology lies in the fact that it forces the applied entomologist to look far more closely into his problem than would otherwise be the case. This may well result in the discovery of weak links through which the species may be attacked. On the other hand, it may do no more than provide a rational explanation of control procedures that have already been arrived at empirically. But even that is a contribution which should not be despised, for only rational or scientific understanding can provide a stable foundation on which a permanent body of practical knowledge can be built up.

Similarly, in the study of insecticides. All the greatest discoveries in this field have been made empirically. But the subsequent study of the physiological factors which control the entry of insecticides into the insect, of the ways in which they bring about the death of the insect or of the physiological mechanisms of acquired resistance to insecticides—all this serves to codify our knowledge of the subject and to put it on a rational basis, and at the same time to point the way to further advances.

That is what I conceive to be the function of insect physiology in relation to applied entomology—and it is a very important one. But there is a debt of perhaps even greater magnitude in the other direction. Many of the most important discoveries in insect physiology, and indeed in biology in general, have resulted from the intellectual stimulus of contact with some practical problem.

INSECTS IN RELATION TO THE ACCUMULATION AND ADHERENCE OF INSECTICIDES

By W. A. L. David, *Agricultural Research Council Unit of Insect Physiology, University of Cambridge*

The details of the process of insecticide accumulation vary according to whether the insect stage concerned is at rest, walking or flying. It is at its simplest in the stationary state. Here a lethal dose for the insect must be deposited directly on each area equivalent to that of the insect unless a fumigant or a spreading liquid are employed. In contrast a walking insect should be easier to kill. As it moves around it accumulates insecticide from an area many times as large as its own target area so that a much less complete cover is necessary, fewer particles are required, it is not so essential that a liquid insecticide should spread and a fumigant action is not so advantageous.

The deportment of the insect and the nature of the environment influence the accumulation and adherence of the insecticide. After the treatment the insect is left standing in a film of insecticide or among scattered particles. When it walks the three legs of one side often come to occupy successively very nearly the same position. Thus the first leg may modify the deposit for the succeeding legs. For example, when a beetle walks through a dust film on a smooth surface it can be seen that the film is piled up into ridges by the slight slipping movements of the tarsi. As the beetle walks more dust is picked up by the legs until they carry large aggregates. These then fall off and new ones form so that after walking a certain distance the beetle loses about as much dust at each step as it picks up.

On a smooth surface the contact of an insect with a surface film is at its minimum and simplest. But even here the relationship is still relatively complex and variable. A long-legged insect may only touch the surface with the tips of its legs but it may also make contact with its mouthparts and antennae. If the insect has short legs its abdomen may be constantly dragged along the surface or it may touch it occasionally or only in special circumstances as when the gut is full of food or the ovaries full of eggs. All these factors will influence insecticide accumulation. On a rough surface contact may be much more extensive and particles may be picked up on areas of the insect which would escape on a smooth surface. Conversely, more insecticide may be rubbed off.

In the case of liquid deposits it is not immediately apparent whether a complete film or scattered droplets would be more effective. A film could be so thin as to contact only the under surface of the feet and tarsal hairs which in some cases may not be easily wetted so that contact is restricted to these sites. If this is so an insect may receive less insecticide from such a film than from a deposit of scattered droplets into some of which it occasionally plunges a foot sufficiently deeply to immerse the last tarsal segment. If the tarsus is wetted the droplet may be blotted up, the leg acting as a wick to carry the insecticide further.

Insecticides are also accumulated by insects in flight. For the impact to occur the insect or the particle must move. When they come together the air movements must not be such as to cause diversion of either in the slip stream around the other. The situation is further complicated because the wing movements of the insect draw in air from the atmosphere which surrounds it. The insect therefore encounters particles from a larger volume of space than it sweeps in its direct flight path.

The movements of the insect in flight may be divided into two categories, the motion of the body and the vibration of the wings. In *Aëdes aegypti* the wings beat about nine times as fast as the body moves through the air. As would be expected in a fine mist (particles 1–10 μ diameter) more droplets impact on the wings than the body since the movements of the insects are responsible for the impact. When the impactions are caused by rapidly moving droplets these differences are largely eliminated. This is so in the case of locusts exposed to droplets 200 μ in diameter. Unfortunately, however, a direct comparison with *Aëdes* cannot be made, as a locust's wing only moves one to three times as fast as its body.

Once the insecticide has reached the insect many factors still influence its insecticidal effectiveness. It is only possible now to consider some of those which apply to dusts.

In the first place it may be noted that visual appearance is a very misleading guide to the weights of insecticidal dusts based on different carriers which are adhering to insects. But in the case of DDT dusts made by solvent application of the toxicant even if the weights of different carrier/toxicant dusts adhering are determined it has been found that these give no measure of insecticidal effectiveness. For this rather unexpected result there are several possible explanations. First, in the case of a DDT-coated particle, only the insecticide on the face of the particle in contact with the insect may be available. The weight of the carrier per unit area of contact surface increases directly as the particle thickness in the plane parallel to the cuticle increases. The thicker the carrier particle therefore the greater the weight of carrier associated with the insecticidal contact surface. Secondly, carriers differ in specific surface areas over a wide range. Thus, if the DDT is largely deposited over the surface of the carriers, dusts composed of small particles will have a low deposit per unit surface area and coarser dusts a higher deposit. Unless the low deposit can supply all the DDT that the cuticle can take up these differences should be significant. Small particles differ from large also because they can cover the insect more closely. Again small particles aggregate and the insect may become covered with a multi-layer of particles of which only the innermost faces of those in contact with the cuticle contribute DDT to the insect. Thirdly, the effective dose may penetrate from restricted sites and therefore gross variations in the quantity of dust adhering will be almost entirely irrelevant as much of it is on relatively impermeable regions. The general conclusion to be drawn from these observations is that the effectiveness of a DDT/carrier dust cannot be deduced either from the total weight of material adhering or even from the quantity of DDT present since varying fractions of the DDT in the dust come into contact with the surface of the insect.

In contrast with the foregoing case we find that with BHC dusts there is a general relationship between the quantity adhering and the kill obtained. Doubtless this is because all the BHC on the insect gives off a toxic vapour which envelops the insect and penetrates via the general cuticle and the tracheae.

In assessing the biological action of a dust deposit on an insect it is necessary to consider how much insecticide is present, for how long it remains adhering, how much of the material present is in contact with the cuticle, how permeable the cuticle is at the point of contact, and how effective insecticide is when it penetrates from a particular site. Many of these factors of course apply to insecticidal sprays, but it appears that with liquids there is greater uniformity in cover and availability of the adherent insecticide than is commonly found with dusts.

THE ROLE OF CUTICLE AND EGG-SHELL MEMBRANES IN THE PENETRATION OF INSECTICIDES

By J. W. L. Beament, *Agricultural Research Council Unit of Insect Physiology, University of Cambridge*

The penetration of an insecticide into an insect or egg involves (except possibly for stomach poisons) the transfer of the toxic agent from the outside of a membrane of stable non-living material to the live substance inside. In the past 10 years, insect physiologists have discovered that these cuticle or egg-shell membranes are extremely complex; their advances have led to much speculation on the physico-chemical processes involved when poisons are transported through these layers.

The general plan of construction of the insect cuticle is derived from Wigglesworth's (1945, 1947) work on *Rhodnius*, and supported by such papers as those on *Tenebrio* (Wigglesworth, 1948), and ticks (Lees, 1947). In these cuticles there is a thin outer layer of cement, consisting of tanned protein, and an underlying very thin wax layer (Beament, 1945). Below the wax are the living contents of the pore canals. Webb & Green (1945) have shown that materials penetrating through artificial systems of membranes built up on this plan behave in a similar way to insecticides, and of course, generally speaking, the useful commercial insecticides appear to have a degree of both water and lipoid solubility and could readily penetrate such a system.

Egg-shells do not appear to adhere to such a regular plan—but those which have been investigated do appear to have a waterproofing wax layer (Beament, 1947, 1951; Davies, 1948; Matthee, 1951), either inside or outside a membrane system which is composed of modified protein layers. However, eggs are usually further protected by a more or less uniform imperforate membrane, secreted by the embryo inside the chorion, and inside the wax layer. It is not necessarily present throughout the whole life of the embryo.

While knowledge of protective membranes has increased our ability to explain insecticidal action, we must take account of a large body of work showing that a substance can have very different toxicity depending on the part of the 'surface' of the insect to which it is applied. Our knowledge of cuticle, confined to the 'generalized' part of a minute, and not particularly representative sample of the insects, gives no indication of the variations in structure associated with the many organs derived from the cuticle in one insect. A little more is known of the effect of structure on penetration for one egg-shell—that of *Rhodnius* (Beament, 1948). The general shell, consisting of some eight layers of modified protein, is virtually impermeable to toxic substances, even with such small molecules as acetic acid or methyl alcohol. But there is ample proof that all toxic materials penetrating these eggs do so either via the true micropyles (Beament, 1947) or through the respiratory layer (Wigglesworth & Beament, 1951). This respiratory layer, deep in the shell substance, though often outside the waterproofing wax layer, completely surrounds the living material in many eggs, and is in free communication with the atmosphere outside, by means of one or more pores. If an egg does not at once succumb to a poison, there may nevertheless be a deposit left in this air space, and it may give rise to toxic effects later in development, if the egg is then exposed only to a suitable solvent; this has been shown using an inorganic salt and water (Beament, 1948). From this evidence it is apparent, not only that a sprayed ovicide must reach an egg, but it must reach a particular part of it, before any penetration can follow. The properties of the walls of micropyles and respiratory canals are vital to successful penetration. But, for the existence of such structures, it would seem that the only way of killing many insect eggs would be to cut off their respiratory supplies by physical means.

Further, we have so far assumed (and a great amount of insecticidal work appears to assume) that cuticles and egg-shells are static structures. This is by no means true. The moulting of cuticle, as Wigglesworth has shown, is not a sudden process, but one which is

preceded and followed by considerable changes in the cuticular membranes extending over many days. There occurs, for example, a time when the protoplasmic filaments are withdrawn from the old cuticle, prior to the secretion of the new one, and from then until moulting occurs, a poison which penetrates the cuticular wax does *not* at once reach living material. In eggs, the detailed changes which take place in the inner membranes (secreted and reabsorbed by the developing embryo) can be correlated very closely with the resistance of the egg to poisons (Beament, 1949). This type of phenomenon must provide a major part of the explanation for variation in toxicity with age, which has been reported in a great many insect eggs (Gough, 1940, etc.).

In conclusion, however great the recent increase in our knowledge of insect cuticle and egg-shell, research has only served to show how crude the picture is, when we consider the fine detail of the specialized structures derived from them. We cannot go much further with the interpretation of insecticidal penetration without knowing the detailed cuticular structure of sense organs, limbs, etc. The few cuticles that have been studied show variation within the general plan, and it would be unsafe to go very far without detailed knowledge of all the commercially important insect cuticles. The same is true to an even greater extent for egg-shells. Their structures are so variable that analogy from one species to the next may be unwarranted. Equally well, physiological studies have shown how vital it is to have well standardized laboratory material for test purposes, for an hour's difference in age can involve the secretion of a new wax layer, and a tenfold increase in resistance to a water-soluble poison. An unfortunate arbitrary choice of age groups could easily lead to anomalous results. But there is hope that an increase in the knowledge of fine structures and the changes occurring during development will allow of the interpretation of many phenomena associated with insecticidal action.

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FEATURES OF THE ECOLOGY AND CONTROL OF THE SHEEP TICK, *IXODES RICINUS* L., IN BRITAIN

By A. Milne*, *Department of Agriculture, King's College, Newcastle-upon-Tyne*

Life history

Ixodes ricinus is a three-host tick. The larva, nymph and (adult) female each need a blood meal. The male usually does not feed but must get on a host to meet the female.

The life cycle is complete in 3 years (Campbell, 1946). The tick spends only about 3 weeks altogether on hosts (MacLeod, 1932). Hence almost its whole existence is passed on the ground.

Distribution as influenced by water requirements

The tick is practically confined to the hills and moorlands (Milne, 1944, 1950*b*). Distribution is correlated with the physical character of the sward, not with the species composition. The tick never gets a hold on the shallow open-textured pastures typical of the lowlands and rarely occurring on hill-land. This has been proved again and again by farmers bringing on infested flocks. Experimentally, engorged ticks have been shown to die of desiccation in such poor cover (Milne, 1950*a*). Where the tick does flourish is in rough pasture, i.e. in the deep cover of bents, bracken and heather, or in the ill-drained environment of rushes. These plants are, of course, the dominants of hill and moorland pastures. Up to a point tick density increases with average depth of cover—other things being equal.

The role of cover is to maintain the rather high humidities necessary for survival (Milne, 1950*a*). To function efficiently, the tick must have access to a micro-atmosphere constantly verging on saturation (Lees, 1946). The deeper the cover the more protection from desiccating sun and wind. Moreover, the hill-pasture dominants all furnish basal layers (mat, litter, or mossy peat) which act like sponges, retaining water even in droughty periods.

The part played by cover is confirmed by results from improvement of rough pastures (Milne, 1948). Such improvement involves, among other things, a considerable lessening in depth and density of sward together with destruction of the basal 'sponge' and tick populations are reduced or practically extirpated according to the degree of improvement.

Micro-habitat economy

One typical micro-habitat is provided by the rough bent pasture, commonly dominated by *Nardus stricta* (with some *Molinia* and *Juncus articulatus*). The living herbage furnishes a dense covering often about 6–9 in. deep with some stems (e.g. *Juncus*) rising to 18 in. Basally, there is a mat of dead and decaying stems and blades perhaps 2 in. or more thick.

At the grass tips, humidity during daylight may fall to 40 % R.H., but at night it always rises to 100 %. In spring (north-east England) it is less than 80 % for about 12 hr. per day on the average. On the other hand, in the upper half of the mat (upper mat), humidity is *constantly* at or very near 100 % throughout the year (Milne, 1950*a*).

The unfed tick, when not active, lies in the upper mat (Milne, 1950*a*). It is inactive for about 6 months after emerging from the egg or moulting (Campbell, 1949). Then it climbs one of the taller living stems and settles down near the tip (Lees, 1948; Milne, 1950*a*) to wait for a host. Immobile, it may wait any time from a few minutes to many days (Lees & Milne, 1951). (Paradoxically, it is now termed 'active'.)

After engorgement, the tick drops back on the ground. Any progression in the horizontal plane is then random and confined to the minimum necessary for finding a way down into the upper mat. There the tick develops to the next stage or lays eggs. Should there be no suitable cover within about 3 in. radius of its arrival point on the ground, then the tick perishes (Milne, 1950*a*).

* Agricultural Research Council Unit of Insect Physiology.

Seasonal activity of tick populations

There are two types of seasonal activity in Britain: the mono-annual or spring type, and the bi-annual or spring-and-autumn type (Milne, 1945*a*). In bi-annual activity the spring-active population is the larger; activity is low at the height of summer and absent in winter. Mono-annual activity occurs on the eastern side of Britain, bi-annual on the western side, including Wales (Milne, 1948).

It has been claimed that, in different parts of Wales, the tick is active in greater numbers in autumn, or active only in summer or only in autumn (Edwards & Arthur, 1947; Arthur, 1948). But these claims arise from taking stock infestation to represent tick activity (numbers on the grass tips) without allowing for effects of delayed or differential stocking (Lees & Milne, 1951).

Individual activity

With no host forthcoming, the average adult tick spends 8–10 days in a season at the grass tips (Lees & Milne, 1951). These 8–10 days are made up of three to four separate phases of 2–3 days each. Including the quiescence periods (in the upper mat) between phases, the active life encompasses about 4 weeks in all. If still unfed, the tick dies very soon after its final appearance at the tips. Hence an individual cannot be active in two successive seasons at the same unfed stage.

The old theory, that the active season comes to an end because all ticks have been fed, was disproved (Milne, 1945*b*) by withholding hosts ('delayed stocking') for the first half (*c.* 6 weeks) of the season; little more than half of the tick population was fed and activity ended at the normal time. Since each day, from the beginning of the season, some ticks become active for the first time, the key to this result is the relatively short active life of the individual (Lees & Milne, 1951). Indeed the seasonal curve of population activity is governed mainly by threshold temperature, time-distribution of onset of individual activity and duration of individual active life.

Spatial distribution

Any mammal or bird coming in contact with the sward is a potential host (Milne 1949). Sheep are practically the only *domestic* hosts in typical tick country. And sheep are the mainstay of tick populations. At least 94 % and probably nearer 99 % of female ticks achieving a host are fed by sheep (Milne, 1949).

The tick depends on a host coming within reach during one of its few short active-phases. Even at one sheep per acre (highest density on hill farms) probably only about one-third of the available females achieve a host (Milne, 1950*b*). Hence host-potential, as well as ground cover, is an important factor in survival.

Spatial distribution, actual and potential (pasture area suitable for ticks and opportunity for their spreading have both increased), can be explained satisfactorily in terms of interplay between the two main factors, viz. amount of ground cover and amount of host potential (and cover is the master factor). Within limits, the better the cover and the higher the host-potential, the greater the density of ticks (Milne, 1950*b*).

Control

Pasture-improvement as a means of control is generally impracticable (Milne, 1948). Withdrawal of the flock (also impracticable) would ultimately improve sward conditions *for* the tick and increase wild hosts. Nevertheless, the most promising line of attack actually does rest on the fact that more than 94 % of female ticks depend on sheep.

Obviously the killing, for 3 years, of all females parasitizing the flock would give a very large measure of control. The major difficulty is that the height of tick activity coincides with the period (11 April to 21 May) when breeding-hill-ewes cannot be dipped (Milne, 1945*c*). The old arsenic-derris dips were effective for about 2 weeks only. It has not so far been claimed that the new DDT or Gammexane dips have solved the problem.

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ASPECTS OF THE PHYSIOLOGY OF THE SHEEP TICK

By A. D. Lees, *Agricultural Research Council Unit of Insect Physiology, University of Cambridge*

Recent work on the ecology of the sheep tick has posed many problems of a physiological nature that could best be investigated by laboratory or by combined laboratory and field studies. Three subjects of relevance to sheep tick ecology are briefly considered in the following paragraphs.

The water relations of *Ixodes ricinus*

The distribution of the sheep tick on the ground led MacLeod (1936) and Milne (1944, 1950) to conclude that the tick can only persist if the vegetational cover is such as to provide humid conditions in the microhabitat throughout the year. This has been tested as described by Milne (above) and the results, in turn, have suggested that the tick is unusually susceptible to desiccation. In comparing *I. ricinus* with fifteen other species of ticks, all of which live in drier environments, the sheep tick has been found to be the least resistant to desiccation (Lees, 1947). Water loss by evaporation is some fifty times more rapid than in the most resistant member of the series; and the eggs are equally susceptible Lees & Beament, 1948).

The dog tick, *I. canisuga*, provides an interesting comparison for, although it lives in the same general localities as the sheep tick, it occupies a different habitat (dog kennels or the earths of foxes). Sheep ticks are dropped here but cannot survive the somewhat drier conditions. *I. canisuga* is about three times as resistant as *I. ricinus*.

These variations in resistance are due to differences in the waxes of the cuticle surface. One indicator of the presence of such a wax layer is the abrupt increase in evaporation which takes place above the 'critical temperature' (Ramsay, 1935; Wigglesworth, 1945). In the above-mentioned tick series, the critical temperature ranged from 32° C. (in *I. ricinus*) to 75° C. As in insects, therefore, the most susceptible species also shows the lowest critical temperature. This and other evidence indicates that it is the relative inefficiency of the wax as a water-proofing agent which limits the distribution of the sheep tick on the ground.

The wax layer serves as a passive barrier against water loss. But unfed ticks, which do not drink water, also possess the faculty of taking up water through the cuticle after desiccation (Lees, 1946, 1948*a*). In this active process, which involves an expenditure of energy, water can rapidly be withdrawn from saturated and even from slightly unsaturated atmospheres. This mechanism may have considerable survival value in nature for the tick must sometimes leave the zone of permanently saturated air, for example when seeking a host in the upper vegetation.

Host-finding behaviour

The behaviour of the hungry tick, in finding and attaching itself to a host, involves a relatively complex series of perceptions (Lees, 1948*b*). When walking up and down grass or rush stems, the active tick attains a position of vantage near their tips by repeatedly turning upwards and finally coming to rest. This is a response to gravity. A response to the humidity gradient is also sometimes seen. When 'in position' several stimuli may provide warning of the approach of a warm-blooded host. Eddies of warm air are important, particularly if the host is advancing from the windward side (temperature sense); any shadow which suddenly falls across the tick is instantly perceived (sense of light intensity); and so are any unusual movements of the vegetation (sense of vibration). The final orientation to the host involves the senses of temperature and smell. The sensilla responsible for the perception of odour, temperature, humidity and vibrations are different types of bristles, mostly located on the forelegs.

After climbing on to the host, the tick still requires a preliminary period of stimulation before it is willing to attach itself. The thermoreceptors and the palpal organ—a contact chemoreceptor—are concerned here.

Tick activity and survival

Newly moulted ticks possess a fixed quantity of reserve substances which cannot be replenished until the next blood meal is taken. These reserves consist of fats which are stored in the gut, the fat body, the Malpighian tubes, the salivary glands and in other tissues (Lees, 1951). The fat depots are drawn upon very sparingly if the tick remains completely immobile (as it usually does if kept in small culture tubes at a constant temperature) but are rapidly depleted once sustained activity begins. Even the disturbance caused by removing the ticks from their tubes three times weekly and then returning them is sufficient to reduce the expectation of life from about 62 to 38 weeks.

This apparently isolated fact has important practical consequences. Observations on the spontaneous activity of a population of female ticks marked individually with coloured paints showed that the active life in nature was also remarkably short—about 4 weeks only (Lees & Milne, 1951). Because of the rapid exhaustion of the reserves during activity the life cycle of spring-fed ticks cannot extend beyond three years. And this fact is also relevant in other contexts, e.g. in explaining the results of the delayed stocking experiments discussed by Milne (above).

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REVIEWS

Comparative Animal Physiology. By C. Ladd Prosser *et al.* Pp. 888. Philadelphia and London: W. S. Saunders and Co. 1950. 63*s*.

The student of Comparative Physiology has for long been handicapped by the absence of a good text-book on the subject. The literature is enormous, and although there are a number of excellent monographs on various aspects of the field even these make up a formidable and expensive array and a sound text was badly needed. The two American books hitherto available deal with the subject phylum by phylum which is perhaps not the most suitable approach; the topic method, followed in this country, gives greater opportunity for stressing the comparative side, but, unfortunately, of the two books published here, one is too elementary, and the other is rather old.

The present American book approaches the subject along 'topic' lines: after sections on Water, and Inorganic Ions, it considers Protein Specificity, Nutrition, Feeding and Digestion, Nitrogen Excretion, Respiration and Metabolism, and Temperature; a series of chapters then consider Photoreception, Chemoreception, Phonoreception(!), Mechano- and Equilibrium-reception and Circulation of Body Fluids; there are then chapters on various effectors, Muscle and Electric Organs, Amoeboid Movement, Cilia, Trichocysts and Nematocysts, Bio-luminescence and Chromatophores and Colour Change; finally there are chapters on Endocrine Mechanisms, and Nervous Systems. The 'sensory' chapters are contributed largely by T. L. Jahn and V. J. Wulf; F. A. Brown jun. is responsible for most of the 'effector' chapters and the section on Endocrines; D. W. Bishop contributes the chapter on Respiration and Metabolism; and the remainder, about three-fifths of the whole book, is written by Ladd Prosser who, as editor, is also responsible for the whole.

Apart from the chapter on Protein Specificity, which is so inadequate as hardly to have been worth while, all the sections cover the ground with great thoroughness. The book attempts to act as a text-book at advanced and early graduate level, and to act as a source book adequate 'to introduce investigators to particular branches of the subject'; the authors also hope that the book may provide 'the physiological material for courses in invertebrate zoology, protozoology, helminthology, entomology, icthyology, herpetology, ornithology, and mammalogy'. It would be a miracle if these purposes were all fulfilled; in fact, the book is so heavily weighted in favour of providing material and acting as a source book, that it unfortunately fails lamentably as a text. A text-book should teach; the book hardly attempts this task although the 'sensory' sections are better than the rest from this point of view. It is true that each chapter has a short introduction and conclusion, but these, generally, are quite trivial; the substance of the chapter is devoted to a very thorough but rather dreary treatment of what has been done, with little attempt to select the significant and exclude the irrelevant. Large sections of the book read rather like a collection of summaries to papers, and are simply peppered with references; there are well over 3000 citations. Even the tables are depressingly extensive with many extending over three pages and, again, little attempt to select even where other authors have already printed most of the material in convenient form; for example, the table 'Non-protein nitrogen excretion in different forms' covers no fewer than five pages yet is itself compiled from comparatively few sources all readily available. Again, Table 9, for 'Concentration of ions in sea water and body fluids' includes no fewer than nine values for sea water itself A source book, surely, should lead one to the source not attempt to be the source itself.

It is not pleasant to have to make these criticisms when the authors deserve our thanks for even attempting such a herculean task. And, after all, though they may have failed to produce a useful text-book, they have undoubtedly succeeded in producing a work of reference of great value to all biologists.

C. ELLENBY

Organisme et Sexualité. By M. CAULLERY. Encyclopédie Scientifique, G. Doin et Cie. 487 pp., 139 figures. Paris. 1951. 2300 francs.

The second edition of Prof. Caullery's book is in many ways comparable to Hartmann's and Haemerling's German texts on similar subjects, but it is of course brought up to date and more concise. No similar work appears to exist in the English language.

A general introduction is followed by chapters on the sexuality of Protozoa and primitive plants and the chemistry and physiology of gamones and termones. The sexual behaviour of Metazoa and higher plants is described in some detail and asexual multiplication, polyembryony, parthenogenesis, hermaphroditism discussed in special sections. Secondary sex characters, sex hormones (mainly of the vertebrates), sex determination, intersexuality and gynandromorphism are described and well documented by examples.

The book is more comprehensive than profound. No new and unifying ideas are developed and some older concepts are omitted, e.g. the demonstration by the reviewer (*Biol. Zbl.* 1932) that morphological anisogamy confers a selective advantage over isogamy and that one aspect of sexual diversity is to consider it as a form of division of labour.

There are 793 references, an author index and a subject index of $3\frac{1}{2}$ pages. H. KALMUS

Embryos and Ancestors. By G. R. DE BEER. 159 pp. Revised edition 1951. Oxford University Press. 1951. 15*s.*

This second edition of de Beer's well-known book presents a great deal of fresh evidence, but otherwise it is 'the previous book brought up to date and enlarged'.

Haeckel's theory of recapitulation is again refuted and in its place other ideas concerning the evolution of animals and to some extent of plants are proposed. Of these the most fertile seems to be neoteny, the concept that larvae of other juvenile forms by becoming sexually mature provide the material for new phylogenetic departures. In such cases it would not be reasonable to expect a recapitulation of the 'old' adult features in the new species; similarities of immature stages of a later form with the adult form of a supposed ancestor are only a consequence of a greater similarity of the larval forms of both: the symmetrical larva of a plaice resembles other 'ordinary' fish, but it resembles their larvae even more. Again man is similar in certain features to the adult anthropoids; he is more similar to young anthropoids. In fact, de Beer regards man with his slow development, his naked body and muscular underdevelopment as an example of neotenic evolution.

Morphological concepts such as paedomorphism and gynandromorphism are discussed and some deviations of peculiar interest analysed, e.g. the development of specially adapted, conspicuously different larvae in certain insects, while the adult forms are more conservative and conforming to type. Some differences between ontogeny and phylogeny, as well as between embryological and genetical concepts are described and the limitations of the germ layer theory shown. De Beer's conclusion is that past phylogeny and present ontogeny have hardly anything to teach each other. However, it is possible by a causal analytic study of present evolutionary processes to collect experimental evidence about the variability and genetics of ontogenetic processes. H. KALMUS

The Association of Ants with Aphids and Coccids. By G. E. J. NIXON. London: The Commonwealth Institute of Entomology. 5*s.*

Not the least interesting characteristics of ants are their relationships with other insects, and to economic entomologists and virus workers their association with aphids and particularly coccids is of considerable importance. In this publication the author surveys the literature primarily from the economic point of view. He has clearly attempted to include as many facts as possible in a limited amount of space, and has avoided as far as possible advancing his own theories on behaviour, which is often an irresistible temptation where ants are concerned.

If it is accepted that one of the most important objects of a survey of this kind is to draw attention to important problems which require further research, then a useful purpose has been served, because this work provides much food for thought. Entomologists in general will find much to interest them.

There is an extensive and valuable bibliography. F. H. JACOB

Soil and Fresh-Water Nematodes. By T. GOODEY, F.R.S. Pp. xxvi + 390, 183 line illustrations. London: Methuen. 1951.

Dr Goodey's book is a clear and comprehensive account of the genera of free-living nematodes inhabiting fresh water and the soil. Some reference is also made to forms occurring in brackish water or soil soaked by brackish water, and to plant parasitic forms in so far as they are also soil inhabitants. A brief introductory section on some of the methods used in collecting and preparing nematodes for microscopical examination and on the general structure of nematodes precedes the main body of the work which is set out according to the system of Chitwood and Chitwood, in their *Introduction to Nematology* except for the Order Tylenchida, which is based on the more recent classification of Gerald Thorne. Each genus is defined and an illustrated description of the type species or some other characteristic species is given. This is followed by an alphabetical list of other species in the genus with their synonyms and by brief comments on their bionomics. Dr Goodey indicates clearly the genera which he considers of uncertain systematic position, and those for which the definition is inadequate or otherwise unsatisfactory. He also draws attention to the genera in which the status of the various species requires revision. The work terminates with an appendix on Hairworms (Gordiidae) and a short bibliography of the more important literature cited.

The substance of the book is largely factual and non-controversial, although some disagreements may arise on points of nomenclature and synonymy. It is primarily a work of reference and as such its outstanding value lies in the bringing together in one focus all the essential information on the known genera of soil and fresh-water nematodes. Nematodes are frequently encountered in the investigation of soil and fresh-water habitats, amongst moss, roots, stems and leaves, and in decaying plant and animal material of all kinds. In the past, the observer has usually failed to identify the species encountered, partly because of the inconvenience of consulting the diffuse literature on the subject. Dr Goodey's book removes this difficulty, and enables a beginning to be made of identification without undue labour. The book should be especially useful to nematologists, ecologists and plant pathologists, as well as to students of zoology in general. Its value would have been enhanced by a more extended treatment of the introductory sections on technique and the general structure of nematodes, and by a larger bibliography. The book is printed on austerity lines and the reproduction of many of the line diagrams fails to do justice to the original drawings. These criticisms are small when weighed against the merits of the book as a whole, and the latter criticism is one of the present times rather than of the author. Dr Goodey is to be congratulated on this solid contribution to Nematology. F. G. W. JONES

'Die Überträger pflanzlicher Viruskrankheiten' (The vectors of plant viruses). By KURT HEINZE. *Mitt. biol. Anst.* (*Zentralanst.*), *Berl.*, Heft 71, 1951, pp. 1–126.

This useful compilation is divided into two sections: first, in tabular form, are listed the insect and mite vectors of plant virus diseases, their geographical distribution, the viruses they transmit, the shortest interval known between uptake of virus and capacity to infect, and some of the more important references, as a guide to the literature. Current names of the vectors are given, together with well-known synonyms; viruses are enumerated under their common German and English names, as well as by the classifications of K. M. Smith and Holmes. Reference is very easy for all workers, whatever classification they favour, as all names and synonyms of insects and viruses are given in indices. In the second section the

viruses are listed under plant families, the known vectors of each virus being enumerated. This work of reference will be of great value to all plant pathologists. L. BROADBENT

Leaf Curl Disease of Cotton. By S. A. J. TARR. Pp. iv + 55, pls. 12, 1 map. The Commonwealth Mycological Institute, Kew, Surrey. 1950. Price 12*s.* 6*d.* or $2.00.

Leaf curl disease of cotton, though very restricted in its geographical distribution (it is confined to Africa and to the Anglo-Egyptian Sudan and Nigeria in particular), can have a devastating effect on a cotton crop, and at one period threatened the cultivation of Sakel cotton in the Anglo-Egyptian Sudan; in consequence the bulk of the research on leaf curl has been done in that country. Mr Tarr's monograph gives a concise and comprehensive account of all that is at present known about this disease and its control.

The monograph is divided into twenty-three sections commencing with a short introduction on cotton cultivation in the Anglo-Egyptian Sudan. The author next discusses the various conditions of cotton that have been described as leaf curl disease, virus diseases of Malvaceae other than cotton, and the distribution, symptomatology, and histopathology of the disease in the Anglo-Egyptian Sudan. This is followed by sections on the transmission of the disease, the life history of the insect vector in the Sudan (*Bemisia gossypiperda*), seasonal carry-over and persistence of the disease, factors influencing its incidence, spread and control, and the breeding of leaf curl resistant cotton. The last two sections are devoted to a discussion on the nature of leaf curl resistance and the leaf curl virus.

It is obvious that a great deal of thought and care has gone into the preparation of this monograph, both in the breadth of its scope, and in the more detailed description of all aspects of the disease including the various cultural methods at present in use for its control. These, as Mr Tarr states, have probably reached a point beyond which no extensive advance is likely to be made. They are, however, laborious and need constant careful supervision, and the author rightly stresses that it is only by the breeding of resistant varieties of cotton that complete control of this disease will be obtained.

The twelve plates of photographs are excellent and greatly assist in the explanation of the text.

The monograph concludes with a bibliography of 113 references. F. W. ANDREWS

Introduction to Mycology. By J. A. MACDONALD. Pp. x + 177, 163 figures. London: Butterworth's Scientific Publications. 1951. 15*s.*

To some, particularly university teachers of botany, mycology is still the systematics of fungi flavoured with phylogenetic speculation. Others still regard mycology and plant pathology as synonymous terms. Mycology is, however, more than systematics, and plant pathology includes much which is outside the province of a mycologist. This 'Introduction to Mycology' is a great disappointment because an introductory text on general mycology is so badly needed and the present work is little more than a taxonomic outline; whilst for the author to note that plant viruses 'have been excluded regretfully' from the book is as remarkable as the inclusion of viruses in the current edition of Bergey's *Manual of Determinative Bacteriology*.

If the author's standpoint is accepted, the text calls for little comment. The writing is on the whole clear and the minor errors of fact, judgement, and typography which have been noted could easily be remedied. The book provides data from which an elementary student could be encouraged to make various integrations, such as the consideration of spore discharge and dispersal, which are fundamental for a proper appreciation of fungi and their activities. Many teachers may be somewhat unwilling to put the book in the hands of a beginner because of the sensationally misleading illustrations. Trials have shown the abstract designs of figs. 138*d* and 139*a*, with or without their captions, to be meaningless to

mycologists and laymen alike. The accuracy of the observation of any student who submitted drawings such as those of figs. 125 and 142 would be seriously doubted and the striking differences between different versions of the same subject, e.g. the fructifications of *Polyporus betulinus* (figs. 115 and 120), *Armillaria mellea* (figs. 17 and 127), and *Phallus impudicus* (figs. 132 and 136*b*), could only puzzle anyone unfamiliar with the objects represented. Finally, the lack of any indication of the scale of the illustrations (*Saccharomyces* cells occur five times (figs. 5, 6, 22, 77*a* and *b*) on as many different scales) and the juxtaposition of diagrams of widely differing magnification is most confusing.

G. C. AINSWORTH

Pesticide Handbook 1951. Edited by D. E. H. FREAR. Pp. 158. State College, Penn.: Commercial Printing, Inc. $1.00.

This publication is a continuation of *Pest Control Materials*, first issued in 1949. It provides a useful guide to the trade names, composition, and the manufacturers of some four thousand products, including dusts, sprays, fumigants, aerosols, repellents, baits, animal dips, moth-proofing materials and wood preservatives, marketed in the U.S.A. and Canada.

J. T. MARTIN

Pflanzliche Infektionslehre. By ERNST GÄUMANN. Pp. 681, with 467 text-figs. and 107 tables. Basel: Verlag Birkhäuser. 2nd ed. 1951. 44.50 Swiss francs or 84*s*.

Gäumann's *Pflanzliche Infektionslehre* was published in January 1946, and a review in the *Annals of Applied Biology* for August of that year led to the production in April 1950 of an English edition under the title *Principles of Plant Infection*; in August 1951, a second Swiss edition appeared.

The new book is 70 pages longer than the original due not so much to new writing as to the addition of 156 text-figures and 17 tables. It may be noted that some of the original text-figures have been omitted or replaced by different illustrations or by new or amended photographs, line-drawings, etc.: thus, chapter 1 contains 83 text-figures in the first edition and 120 in the second, but although only 37 text-figures have been added, 47 of the illustrations are new. Further, the adoption of a finer, whiter paper in the second edition has permitted greatly improved reproduction of half-tones and the book is now an unusually well-illustrated volume. The Bibliography comprises the names of over 800 authors and is remarkably international: it has been rearranged more conveniently in column form and includes many citations of the year 1950, even one of 1951. The Index, which was inadequate in the first edition, has been greatly amplified and is now almost worthy of the book.

The general structure and format remain unaltered and, apart from changes in four minor headings, the detailed Table of Contents is identical in both editions. The entire text has been revised, minor and not-so-minor emendations occur on almost every page, and certain obvious errors in the first edition have been corrected. Major changes, some of which have involved a substantial amount of new writing, mostly concern recent advances in knowledge of antibiotics, the nature and transmission of viruses and the relation of virus to host, the nutritional physiology of bacterial and fungal pathogens, and substances in host cells antagonistic to invaders. The nomenclature of the micro-organisms referred to in the text has been brought up to date, but English and American readers will still meet well-known fungi, etc., under unfamiliar names.

In a Prefatory Note to the English edition I said: 'I doubt whether any botanist or plant pathologist reading Professor Gäumann's volume could agree with everything it contains, and it is a measure of the originality and independence of the author's mind that every reader of his book will wish to argue one or another point with him. . . .For a generation to come botanists and plant pathologists will find this book not only a mine of information but a source of inspiration, a true "Pierian spring"; but they will also find it a mine of argument

and controversy and a source of endless discussion and questioning—which is what a book of this kind should be.' These words were written of the first edition but they apply, perhaps with even greater force, to the second.

How does the new book compare with the old, not only from the reader's standpoint, a pertinent question in view of the high price of the volume, but in relation to more general scientific values? In my opinion the primary importance of Gäumann's book lay in the fact that it was a masterly contribution to theory; it was a creative formulation and exposition of the principles of plant pathology as a science. That it was also a brilliant selection and a logical marshalling of pathological facts and illustrations was of secondary importance. In the new volume this secondary value is clearly enhanced—there are more facts and more illustrations, and they are more up to date—and, therefore, as a source book of interesting and useful pathological data the new edition is by so much the better. But I am far from sure that this enhancement of a secondary value has not adversely affected what, to me, is the basic value, the blue-printing of skeletal structures, patterns and relationships. The principles formulated in the first edition are identically those laid down in the second edition but, in the former, they stood out starkly with just the right covering of flesh and garments to suggest contours whereas, in the latter, the flesh has become a little too fleshly and the garments too numerous and distracting. The author has, I think, succumbed to the lure of a bigger, better book whereas it was largely the very economy of the original work that gave it its unique value. But this new edition is a lovely book.

WILLIAM B. BRIERLEY

A Dictionary of Biology. By M. Abercrombie, C. J. Hickman and M. L. Johnson. Pp. 246. Harmondsworth: Penguin Books Ltd. 2*s*.

The authors say that their 'aim is to explain biological terms which a layman may meet when reading scientific literature, and to define the terms which a student of biology has to master at the beginning of his career—the thousand or so words which so grimly guard the approaches to science. The entries are not restricted to a bare definition, which, however valuable in keeping one to the narrow path of customary usage, can be most unhelpful in the task of understanding the word in its context. Some information about most of the things named is given, so as to convey something of their significance in biological discussion—to add a faint flavour of a pocket encyclopaedia.'

There seems no better way of reviewing this book than to quote the above passage, and then say that the authors' aim has been brilliantly achieved and that their description of the book is a series of modest understatements. There are nearer 2000 than 1000 entries, many of which are certainly far from 'bare definitions'; indeed, they are model summaries of current knowledge and contain a wealth of information expressed simply and lucidly. The 'flavour of an encyclopaedia' is much more than 'faint', and some of the summaries of half a page or so are as informative as the whole chapters that are often devoted to the same subjects in text-books. The book, too, will be valuable to many more than the 'layman' and 'student at the beginning of his career'. What a boon to the harassed teacher to find all awkward questions answered authoritatively but not dogmatically, and to the research worker who has forgotten, or never knew, or thought he did but was wrong. And all for 2*s*. in 1951: never before can so much have been offered to so many for so little. The authors and publishers are to be congratulated and thanked for a publication that all biologists can afford and few can afford to be without. It seems ungrateful to suggest any change, but the words defined would stand out more clearly if printed black in small capitals instead of being italicised. The dictionary will be in constant use and, though at this price is readily replaceable, many people would probably willingly pay more for a more durable edition. Certainly at several times the present price the contents would still be excellent value for money.

F. C. BAWDEN

Soviet Genetics. By A. G. MORTON. Pp. 174. London: Lawrence and Wishart. 1951. 15*s.*

In introducing this book the publishers have lavished their care on a task which no other profit-making enterprise in the western world would probably have been willing to undertake. The book is devoted to what the author calls Soviet Genetics. There is nothing in it with which the Central Committee of the Communist Party, after having suppressed genetics in Russia, would not agree. There is also nothing in it with which anyone who had contributed to the advancement of genetics would not disagree. The writer's own contribution to this subject is limited to the present work. He has done nothing before and he is not likely to do anything again in this direction. His labour seems to have consisted in the copying out of the wisdom of Woodger, Waddington, the Webbs (Sydney and Beatrice), interrupting these extracts by lengthy cuttings from the works of Engels, Lenin, Michurin and Lysenko, and adding a flavour of diversity to the whole hotch-potch by further excerpts from Huxley, Hinshelwood, the present reviewer, and *The Economist* (a London weekly newspaper). In these quotations—so far as they touch on Genetics—the copyist is out of his depth. But he never notices it. At the end however he almost emerges from a cloud of other people's ideas to declare himself in a flash of dazzling irrelevance: 'From each according to his ability: to each according to his needs,' he blissfully recites. How wise indeed! But in turning to more serious duties the reader may pause to ask himself: then why must Dr Morton write a book on genetics?

C. D. DARLINGTON

Gall Midges of Economic Importance. Vol. v. *Gall Midges of Trees.* By H. F. BARNES. Pp. 270. London: Crosby Lockwood and Son Ltd. 1951. 15*s.*

Gall midges as a group are among the most specific of insects; the plant and the midge may often be said to live an almost symbiotic existence. This close relationship of midge and host has inevitably led the author of these volumes to study relationships of plant to plant as well as midge to plant. The whole question is admirably discussed in Dr Barnes's preface to this, the sixth volume to be published.

Although gall midge larvae do not consume appreciable quantities of their host plants the indirect damage caused by malformation and proliferation of branches and twigs may be considerable, and it is this kind of damage to young trees which is of greatest economic importance. Eighty-five pages are devoted to the midges of conifers and 107 pages to those of broad-leaved trees.

The value of this volume as a reference book may be judged by the fact that there are twenty full pages of references and thirty-eight pages of indexes comprising a midge index, a plant index and a general index. In the words of Dr Neil Chrystal, who writes the foreword: 'Dr Barnes has placed a reference book of great value in the hands of foresters, arboriculturists and all others interested in trees. Knowledge accumulated by many workers over many years, in all parts of the world, has been synthesized by him into the space of a single volume. It is for the readers of the work to take advantage of the rich harvest which the author has gathered for them.'

I. THOMAS

Four Thousand Million Mouths. Scientific Humanism and the Shadow of World Hunger. Edited by F. LE GROS CLARK and N. W. PIRIE. Pp. 222. Oxford University Press (London: Geoffrey Cumberlege). 1951. 12*s.* 6*d.*

This collection of individual essays by a number of distinguished workers in the applied biological sciences provides the thoughtful layman with accounts of some of the ways in which science can assist the world's rapidly increasing population to obtain its food supply. The book is not comprehensive: it contains no information on the present distribution of the

world's population, or on regional population trends, or on regional potential food resources. All consideration of such matters as land-tenure, radically affecting the full application of scientific method to the relief of human need in many parts of the world, is rather too carefully evaded. There is no essay on modern agricultural machinery; and none on our warfare against the legions of insect pests. Indeed the whole collection of essays is more of a nosegay than a Novum Organum. But each essay is informative, and each has an added interest in that it reveals a scientific personality. 'Each contributor has written more or less as he wishes so that his attitude of mind can be conveyed fully to the layman.'

John Hammond, in the full exercise of this freedom, scorns to contribute anything but a straight scientific paper, and that of his best, on 'The Pig as a Producer of Food'. The reader inclined to share W. H. Hudson's view of the pig as a friendly animal, closely akin to mankind, may find this essay bordering upon the horrific, but the exposition is crystal-clear, for butchers and browsers alike. H. D. Keay adopts something of the same attitude towards the cow, in 'Improving the Milk Supply', but with a wider concern for the complex changes in agricultural and national economies that would be necessary to extend the world use of bovine milk in the human dietary. F. C. Bawden in 'Growing Healthier Crops' draws attention to the great losses of produce that are caused by plant diseases, and says plainly that if we want the world's agriculture to feed more people, we had better have a few more plant pathologists *in the field*, even at the expense of a few less workers in the homicidal industries. C. E. Lucas in 'Harvesting the Waters' contributes a particularly satisfying piece of informal but honest biological writing, truly 'of fish and of men', and here, as in the next essay by G. A. Reay and C. L. Cutting on 'The Preservation and Use of Fish', we see the biologist at work, shoulder to shoulder with the fisherman and the engineers, which may seem to some of us to be the highest manifestation of 'scientific humanism'. Somewhere about the middle of the book, in 'Genetics and the World's Food', S. C. Harland introduces some pleasant flights of imagination. After describing actual methods and achievements of plant breeders he soars on to consider what *might* be done if we could but collect hundreds of varieties of our crop plants from every part of their geographical range, cross everything with everything else, and put out the whole hybrid mixture in all sorts of 'ecological niches'. G. V. Jacks in 'Conserving the Soil' shows the sober part played by the natural philosopher, sifting evidence for principles. Setting aside all panic talk about soil erosion, he deals broadly with the greater issue of soil conservation, in relation to plant ecology, climate and human civilization. In 'Manuring for Higher Yields' by F. Yates, there is the same philosophic concern for principles, this time finding its practical expression in the accountancy of fertilizer responses. In 'The Processing of Food', A. L. Bacharach and T. Crosbie-Walsh discuss methods of food preservation from Appert to quick-freezing and add a little about ways of disguising wholesome but new and strange food products to overcome the prejudice of consumers. In 'The Circumvention of Waste' N. W. Pirie shows us how a discoverer lets his thoughts range about a new and potentially important idea, almost before its conception. He considers the possibility of extracting edible protein from the leaves of plants, perhaps more efficiently than farm animals now do it, and has an eye on such materials as pea vines and potato haulms as possible sources of protein-cake for human consumption.

An assertion, in the introductory chapter to this book, that we face starvation 'unless we transform our masses of illiterate and unenlightened peasants and cultivators into alert co-operative farmers capable of exercising a measure of control over their own fecundity' is left in the grandeur of its own ineffability. Apart from an interesting historical review of views on Malthus and Malthusianism by F. Le Gros Clark ('The Malthusian Heritage') the essays all deal with food and not with fecundity. The practice of obstructed copulation, which we call 'birth control', is mentioned hopefully from time to time as a means of reducing the number of mouths to be fed; but there is no biological consideration of the practice or of its psychological and genetical consequences.

E. C. LARGE